THE CHEMISTRY OF BLOOD COAGULATION

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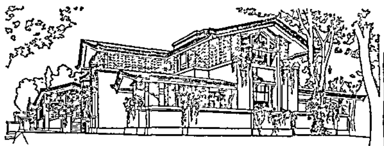
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THE CHEMISTRY
of
BLOOD COAGULATION

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PREFACE TO A TRANSLATION OF
Die Chemie der Blutgerinnung
BY PAUL MORAWITZ

THE NEED to translate a monograph which is chiefly of historical interest and already available in a modern language may be questioned. This monograph provides historical background, with particular emphasis on the 19th Century developments, for the so-called classical theory of blood coagulation promulgated by Morawitz, Fuld, and Spiro fifty years ago. The scientist will gain true wisdom regarding his field of interest and appreciation of its relation to the total knowledge of man and his environment only through familiarity with the development of ideas in his field, i.e., its history. When the worship of the quantitative and of the ever-expanding nomenclature is stripped away, it appears that the basic thought behind much of the current research on blood coagulation had its origin in the hypotheses of the late 19th Century. A fair portion of today's "original" work in this field seems to be a quantitative re-definition of careful observation and thoughtful conclusion made by men of that period.

The progressive decrease in linguistic proficiency of the present-day American scientist and the ponderousness of scientific German amply justify the translation of an important monograph such as this. Some American investigators in the field of blood coagulation admit that they have read but portions of Morawitz' article in the original.

The authors are indebted to Dr. C. Lockard Conley of the Johns Hopkins Medical School for the initial sugges-

tion to translate this work. Appreciation is also expressed to the United States Public Health Service for the support of research projects of the authors which in turn provided both stimulus and background for the translation of this monograph. The authors are grateful for support from the Brownlee O. Currey Memorial Fund.

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INTRODUCTION

THE BIBLIOGRAPHY represents an attempt to gather and categorize widely scattered information concerning the chemical processes involved in the coagulation of blood. Although the collection is brought up to date through 1901 and includes a few references to publications of the following year, it is not necessarily complete. Older studies, because of their limited value for the development of the modern theories of coagulation, have been mentioned only insofar as it appeared necessary within the framework of this monograph. Extensive references to earlier theories can be found in particular in the works of Schroeder van der Kolk (10) and Nasse (8). Furthermore, the vast literature dealing with the role of the formed elements of blood in coagulation will not be discussed in detail except as it appears necessary for an understanding of the chemical processes involved. Detailed information regarding the role of the formed elements can be found in the bibliographies of Loewit (478), Ziegler (12), and Schwalbe (485).

CONTENTS

THE CHEMISTRY OF BLOOD COAGULATION

Chapter I

THEORIES OF BLOOD COAGULATION BEFORE ALEXANDER SCHMIDT (1861)

IT IS quite natural that such a remarkable phenomenon as the coagulation of blood has since ancient times stimulated attempts to penetrate the mystery of its origin. It is no wonder that since the beginning of scientific research there has never been a dearth of hypotheses and theories designed to explain the coagulation phenomenon. The days of humoral pathology, in particular, produced an exceedingly large number of observations in this field. This appears quite understandable since at that time a prominent role in pathology was ascribed to morphological alterations in the fibrin clot. It was hoped that considerable insight into the pathogenesis of disease could be gained by a study of the changes in the blood, especially of the protein and fibrin contents. These ideas persisted well into the last century and were still emphasized in an interesting study by Wunderlich (40).

The numerous theories of blood coagulation which had been promulgated prior to the middle of the last century can be divided into two main groups. The first embraced those which began with the assumption that blood remains basically unchanged while it coagulates but solidifies because of environmental changes. The second group, on the other hand, comprised those theories which invoked chemical rather than physical changes as responsible for coagulation. The workers who adhered to the latter idea theorized that coagulation is either accompanied by a loss of one or

several substances from the blood, by a combination of such substances, by precipitation of such substances from the blood, or by reactions of one or several substances upon others. In short, they believed that chemical processes were the cause of the precipitation of a new, previously nonexistent substance of low solubility, i.e., fibrin.

Among the physical influences to which coagulation could be ascribed, cooling seemed to be the most obvious, especially since a casual observer might readily discern a parallelism between the freezing of liquids and the coagulation of blood. Hippocrates (27) and Aristotle (14) attempted to explain coagulation as a process of solidification upon cooling. This ancient theory, in addition to numerous others, has still been occasionally invoked in recent times and found proponents in Schroeder van der Kolk (10) in the 17th Century and later in Bohn, Hoffmann, and Ludwig. As late as 1760 Butt (20) attempted in a very thorough dissertation to explain the various manifestations of coagulation as effects of different degrees of cooling. It was Butt's idea that blood could be compared to a supersaturated solution of fibrin which partially crystallized upon cooling. William Hewson (26) was the first scientist to prove once and for all that cooling is not responsible for coagulation when he provided evidence that blood can be frozen and, after thawing, liquifies before it coagulates.

Another physical explanation for coagulation was the cessation of the natural flow of blood. A popular hypothesis assumed the existence in the blood stream of an accumulation of particulate matter which was widely dispersed by the circulation but which would quickly aggregate with the cessation of blood flow. For this reason in the older literature coagulation is frequently referred to as "separatio" meaning separation of the solid elements

from the liquid portion. Schroeder van der Kolk (10) alluded to Pechlin, Boerhaave and Van Swieten as proponents of this line of reasoning. Albrecht von Haller (24) also postulated a mutual attraction of the blood cells which was vitiated solely by the constant rapid motion of the blood stream. Schroeder van der Kolk (10) subscribed to similar theories. According to him, the liquid state of blood was not a natural one but was the result of rapid movement, since he believed that oxygen obtained from respiration would otherwise tend to precipitate fibrin in a crystallized form.

In answer to this argument John Hunter (29) stated convincingly that lack of motion cannot by itself be the cause of coagulation since blood can remain stagnant for a time in the vessels without clotting, while stirring the blood did not hinder coagulation but actually accelerated it. Hunter also called attention to the simple observation that while menstrual blood remained liquid, exudates could clot *in vivo*. Thus he questioned the theory that coagulation was brought about by the contact of blood with air. It was the belief of some scholars that contact with air, or for that matter, contact of blood with any foreign body, would initiate coagulation. In this connection Schroeder (10) cited the opinions of Mercurialis and Helvetius. Hewson (26) attempted to support this theory experimentally.

In the same category belong those theories which considered the solid state of blood the natural one and ascribed the liquid state of blood in the body to a "living force." Whenever these theories were not simply glorified ignorance, they led to the conclusion that coagulation was caused by a change in the physical contact of the blood. Hunter (29), although he had originally denied the effect of contact with air, said later, "Where there is a full power

of life, the vessels are capable of keeping the blood in a fluid state."* Hunter found many disciples. Schroeder (10) singled out the following: Levison, Dumas, Sprengel, Burdach, Parmentier, and Deyeux. While Hendy, Blumenbach, Kreysig and Roose opposed this theory, they were unable to substitute a better one. Subsequently Bruecke (17) evolved a theory which as will be seen later related in some respects to that of Hunter.

The second category of theories concerning coagulation, the chemical one based on some transformation of a substance, also has its roots in antiquity. It was Galen (21) who put these ideas into their most universal form, saying, "The formation of a coagulum is the road to decay." Harvey (25) stated more specifically that the various parts of coagulated blood are formed by decomposition and decay due to death and do not exist in living blood. By contrast it should be mentioned in passing that there was no lack of effort to interpret coagulation as the last vigorous manifestation of life in the blood. There was vast disagreement as to the details of the decomposition of the blood and the subsequent formation of an insoluble substance. These differences have not yet been entirely clarified. Home (28) and later Prévost and Dumas (35), proposing a morphological rather than a chemical approach, borrowed from previous theorists the idea that the formation of fibrin was caused by the aggregation of blood cells. Blood cells, according to this hypothesis, consisted of spherical bodies with a pigmented investing membrane and a fibrin nucleus. The nuclei tended to agglutinate but were prevented from doing so by the interposition of the investing membrane. This membrane was more or less damaged

* *Translators' note:* This quotation was taken from Hunter's original work in English (*A Treatise on the Blood,* Vol. I, p. 40, 1812. Printed for E. Cox & Sons, London) rather than re-translated from the German.

in decomposing blood whereupon the pigment escaped into the plasma, and the remaining naked nuclei succumbed to mutual attraction, aggregated, and formed fibrin. This theory persisted for a long time, even though men like Berzelius (15) and Johannes Mueller (33) had disproved it. Not so long ago Mosso (143) still defended *the belief that fibrin was a product of the red cells, and* even recently the origin of fibrin from extravascular decomposition of blood has been defended, particularly by morphologists (e.g., Buerker [460]).

Johannes Mueller's famous and oft-quoted experiment in which he proved the existence of fibrin in solution in the plasma consisted of obtaining a typical fibrin clot from the clear filtrate of a mixture of frog blood and concentrated sugar solution (33). Mueller did not explain how the transformation of the dissolved fibrin into a solid substance was supposed to take place. Other less reticent observers came forth with the most diversified and often contradictory explanations. Thus statements were made to the effect that acid formation (especially carbonic acid), oxygen, ammonia, etc., influenced coagulation in an important manner. Some of these theories are of fairly recent vintage. Even many years after the investigations of Denis (102) and Alexander Schmidt (41, 42) had put the study of coagulation on a sound experimental basis such obsolete ideas appeared again and again (see Matthieau and Urbain [31, 32]).

Finally, the effect of oxygen was emphasized by Virchow himself (39). However, he did not restrict himself to this comparatively unattractive idea, but made considerable progress through careful analyses which led him to conclude that fibrin could not exist in fluids in an isomeric liquid state but rather existed in a precursor state with entirely different characteristics, as "fibrinogen." Virchow

stressed the importance of careful investigation of exudates which he felt would yield important data for an explanation of coagulation. As will be seen later, Virchow's idea was espoused with greatest success by Alexander Schmidt. But Virchow was not the first to recognize the importance of studying exudates in relation to the coagulation problem. Several years earlier Buchanan (19) demonstrated that soluble fibrin* showed no tendency whatsoever to coagulate, but could exist outside the body in a liquid state, and that coagulation was brought about by the addition of another substance. Buchanan experimented with hydrocoele fluid which remained completely liquid by itself and did not coagulate until serum or a clot was added. According to Buchanan it took two substances to cause coagulation: liquid fibrin which by itself was stable, and a substance capable of affecting the fibrin and transforming it into an insoluble form. This shows how closely these ideas resembled the later coagulation theories of Schmidt. Here were concepts which were later to constitute the basis for modern thinking in this field. Buchanan also attempted to fathom the nature of this second mysterious substance which transformed soluble fibrin into an insoluble form. He found it in serum even after all blood cells had been removed. This led him to conclude that under certain circumstances this substance could be found in a soluble form in serum. Numerous other experiments, however, had already apparently convinced Buchanan that the clot-promoting substance did not originate in serum but rather derived from the formed elements of blood. Buchanan incriminated the white cells as the probable source of this critical substance since he showed that in blood allowed

* *Translators' note:* The term "soluble fibrin" in this instance probably implies unaltered "fibrinogen" rather than any intermediate product in the conversion of fibrinogen to fibrin.

to sediment and coagulate that portion of the clot which contained the white cells possessed a particularly strong clot-promoting activity. Pus cells and a number of different tissues had a similar effect.

Accordingly, Buchanan deserves consideration as the actual founder of the modern theory of coagulation. However, the merits of his successors are no less great since Buchanan's discoveries remained generally unknown or aroused little interest. Alexander Schmidt arrived independently at conclusions similar to those of Buchanan.

In addition to Buchanan's work two important studies of the same period should be mentioned since they helped to prepare the basis for the modern theory of coagulation. These are the publications by Bruecke (17) and Denis (102, 103).

Bruecke demonstrated the importance of the vascular wall in maintaining blood in a liquid state. He succeeded in keeping the blood in the heart of a turtle liquid for several days by means of low temperatures. As soon as the blood established contact with foreign substances it coagulated quickly. How could this finding be explained? Bruecke did not wish to give a dogmatic interpretation. However, he suggested that the vascular wall should not be thought of as simply having nothing whatsoever to do with the blood, but that it acted in some unknown manner as an impediment to coagulation. Bruecke's ideas concerning the process of coagulation itself were less productive since he made the assumption that there was no precursor of fibrin in plasma and that part of the protein content of plasma, the so-called "albumin," was precipitated in the form of fibrin by some unknown process, perhaps by the effect of an acid.

In contrast to Bruecke's approach Denis (102, 103) actually attempted to isolate Virchow's hypothetical fibrino-

gen (39). He did this by means of the salt precipitation method which later became very useful for the study of coagulation. As far as the writer has been able to ascertain, Denis was the first to use this method. He collected blood in 1/6 volume of a saturated solution of sodium sulfate thus preventing coagulation, allowed the cells to sediment, and filtered the resulting salt plasma. Saturation with sodium chloride produced a protein precipitate which was washed with sodium chloride solution and dissolved in water. The colorless solution of this protein which Denis first named "serofibrin" (and subsequently "plasmin"*) coagulated spontaneously after a short time, yielding fibrin. The resulting fibrin did not always show identical properties. Hammarsten (123) pointed out later that these differences were due to contamination with varying amounts of "paraglobulin"† or of leucocytes (see below). Nothing was said concerning the changes which account for the transformation of "plasmin" (or as Denis occasionally called it, fibrinogen) into fibrin.

A summary of the status of the blood coagulation problem around 1860 shows that a generally accepted theory did not exist. None of the theories which had been promulgated up to that time had been borne out by facts. It was known, particularly as a result of the investigations of Bruecke (17), that coagulation was not accompanied by a loss of gaseous matter or by absorption of substance from the air. Coagulation had to be investigated on the basis of internal factors in the blood itself which were still unknown.

Few positive findings compensated for these negative

* *Translators' note:* At present the term "plasmin" is used with an entirely different meaning referring to the plasma proteolytic (fibrinolytic) enzyme.

† *Translators' note:* This was later given its modern name of "serum globulin."

results, but they constituted the basis for the modern theory of coagulation. They included Denis' proof of the existence of a soluble precursor of fibrin (fibrinogen or "plasmin" [102, 103]), Bruecke's emphasis on the deterrent effect of the normal, living vascular wall (17), and the results of Buchanan's research (19) which had, however, been largely neglected.

At this point began the epochal studies of Alexander Schmidt who created, in the painstaking labor of decades, the basis for the modern coagulation theory. It must be admitted that Schmidt was not always right, and that he sometimes clung stubbornly to his theories with little regard for the findings of others. This matters little in view of the fact that, in addition to numerous details whose importance was only properly assessed by later generations, his name stands for two fundamental discoveries: the recognition of the importance of the blood cells in coagulation, and his proof that coagulation is a fermentative (enzymatic) process. The first of these seems nowadays practically self-evident. In those days, however, the study of coagulation was still greatly influenced by the ideas of Johannes Mueller (33) who ostensibly had shown that blood cells were unnecessary for coagulation. Schmidt's teachings were a novelty and constituted marked progress. The discovery of "fibrin ferment"* has to be rated no less highly, although Schmidt's findings in this respect had antecedents in those of Buchanan and Bruecke, both of whom emphasized the similarity between the coagulation of fibrin and the curdling of milk by rennin.

* *Translators' note:* The interpretation could also be "fibrin enzyme." Schmidt later gave this substance its modern name, "thrombin."

Chapter II

DEVELOPMENTS IN THE STUDY OF COAGULATION BEFORE 1890 (THE STUDIES OF SCHMIDT AND HAMMARSTEN)

IN HIS FIRST publications of 1861 and 1862 Alexander Schmidt (41, 42), like Buchanan (19), dealt with observations on transudates to which blood serum was added. It was recognized that *some* transudates could coagulate inside the body or clot shortly after their removal from the body. By contrast other transudates, especially hydrocoele, peritoneal, and pericardial fluids were known to remain completely liquid outside of the body. It was believed that these fluids like blood serum lacked the coagulation substrate, and therefore they were referred to as "serous" fluids. Schmidt pointed out that these serous fluids upon the addition of blood serum formed a typical clot, i.e., they contained a substance which by itself remained liquid, but which quickly changed into typical fibrin in contact with a substance present in blood serum. Schmidt called these two hypothetical substances "fibrin generators," and following Virchow (39) he named the protein which is found in the transudates, fibrinogen, and that present in serum, "fibrinoplastic substance." The next project was the isolation of these two agents. The "fibrinoplastic substance" was obtained from highly diluted blood serum by precipitation with CO_2. It was shown that the fibrinoplastic activity was present solely in the protein precipitate while the serum filtrate or supernatent was totally

inactive. It was also possible to isolate from "proplastic" serous cavity fluids (i.e., those which contained fibrinogen but did not clot spontaneously) a protein which yielded fibrin when mixed with the "fibrinoplastic substance" of serum. This protein was isolated by dilution of the serous cavity fluid with water and subsequent acidification, by dialysis, or by precipitation with sodium chloride. Schmidt's reflections concerning the nature of the "fibrinoplastic substance" led him to look first for an enzyme since in the coagulation of transudates with blood serum all the fibrinogen was consumed but never all of the "fibrinoplastic substance." The fluid which was squeezed out of the clot after coagulation in such an artificial system could, like serum, bring about coagulation in other fluids that contained fibrinogen. However, Schmidt soon abandoned this theory. Experimentally the low potency of this "pseudo-serum," the possibility of exhausting the supply of "fibrinoplastic substance" and the relationship of the amount of the latter to the amount of fibrin formed, appeared to him to disprove the enzymatic nature of the process.

On the basis of these and many other studies Schmidt proposed his first coagulation theory. He hypothecated that coagulation resulted from the contact of fibrinogen with the "fibrinoplastic substance." This reaction was viewed as a chemical combination of the two substances rather than a precipitation of one by the other. The preexistence in the circulating blood of a soluble fibrin was denied; fibrin must have been the product of two originally separate substances. Transudates remained liquid because although they contained fibrinogen, they had no "fibrinoplastic substance." There was no need to ascribe the divergent coagulation patterns of blood and lymph to basic differences in the fibrin as Virchow (39) had

thought. Fluids with low cell concentrations possessed only a limited tendency to coagulate. This led to the assumption that the blood cells must play an important part as carriers or producers of clot-promoting substances. This assumption was supported by the demonstration that defibrinated blood brought about a much more rapid formation of fibrin than cell-free serum. The addition of a pure solution of hemoglobin promoted the same acceleration of coagulation. It was thus thought that the "fibrinoplastic substance" probably originated from the formed elements of blood. This theory made it somewhat difficult to explain the liquid state of blood in the body. Schmidt could only formulate hypotheses in this respect. He emphasized various possibilities such as the inhibiting effect of alkali, or changes in one or both of the "fibrin generators" during or immediately after their formation from the formed elements of blood. These studies did not mention the possibility of rapid disintegration of the cellular elements in shed blood.

Schmidt's facts could not be denied. Nevertheless, opposition soon arose to some aspects of his theory. One can disregard the arguments of men like Eichwald (107) who resorted to obsolete ideas in ascribing once again an important role in coagulation to CO_2. Bruecke's criticism was more important since he questioned the identification of the CO_2-precipitated protein ("paraglobulin" or, as it was to be called later, "serum globulin") with the "fibrinoplastic substance" (18). Bruecke expressed the opinion that the "fibrinoplastic substance" might only be adsorbed on "paraglobulin" since a precipitate of the latter did not have materially greater clot-promoting activity than serum in which the protein was found only in a very diluted form. A truly pure "paraglobulin" could possibly have no fibrinoplastic effect. It might be advisable to look for an enzyme as the active substance. Alexander Schmidt did

not remain deaf to these arguments. Further research convinced him that his theory could no longer stand in the light of experimental findings. He dropped it completely upon his discovery of "fibrin ferment"* (44). According to Schmidt there was, in addition to the two "fibrin generators" which could combine in varying proportions, a third substance needed to produce coagulation. This was not part of fibrin and not used up during coagulation. The following considerations led Schmidt to assume the existence of such a third substance and its enzymatic nature. While it was impossible to produce "fibrinoplastic substance" completely free of enzymatic activity, fibrin ferment free of globulin could be produced, and the latter was capable of initiating or at least accelerating coagulation in appropriate fluids, e.g., in diluted salt-plasma or cooled plasma. Numerous reasons were given supporting the enzymatic nature of this substance:

1. Any small amount of it could produce a complete precipitation of fibrin during coagulation.
2. After coagulation the artificial serum contained unused ferment which could be utilized over and over again to bring about coagulation.
3. The substance lost its activity when heated to 100°C. Its optimum activity was at about 37°C. Low temperatures decreased its activity.
4. Alkalis and acids reduced its potency. Neutral salts in low concentrations increased it.
5. It was possible to produce a strong ferment solution which contained only a fraction of organic matter and was almost free of protein.

Schmidt (44) prepared this ferment by the addition of several volumes of alcohol to blood serum. In due time the protein was *completely precipitated without altering*

* See footnote page 11.

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and sedimented horse plasma always began at the leucocyte layer, while the plasma above this layer and the layer of red cells were still entirely liquid. Observations on chyle, lymph, etc., i.e., on fluids which did not contain red cells but coagulated spontaneously, also provided evidence against the participation of the red cells in the formation of fibrin ferment. The red cells, however, played a different role in coagulation since their hemoglobin greatly accelerated coagulation as a catalyst (see the later studies of Bojanus [58] and Sachssendahl [84]). The extravascular disintegration of the white cells led to the formation of fibrin ferment and in addition "fibrinoplastic substance" which was not found in circulating blood except in small traces. The manner in which the two "fibrin generators" combined under the influence of the ferment to form fibrin remained entirely unexplored. It may have been a case of combination of the two substances with a splitting out of the elements of water, or the molecule of the "fibrinoplastic substance" might not have entered into the fibrin complex at all. *This much appeared certain: There was* an intermediate product, a so-called "soluble fibrin" which was precipitated only through the influence of alkali salts, possibly because of the removal of water. Therefore, as Schmidt's disciple, Kieseritzky (71), showed, there was a parallelism between the coagulation of fibrin and that of colloidal silicic acid. In addition to the "fibrin generators" and fibrin ferment the presence of a quantity of neutral salts was also considered necessary for coagulation. A large salt concentration prevented coagulation (see the investigations of Denis [102] and Gautier [22]). The collection of blood in solutions of magnesium sulfate, sodium sulfate or sodium chloride was therefore one of the chief methods for the production of a solution which reacted with fibrin ferment.

This theory contained one weak point, to wit, the parti-

the subsequent solubility of the ferment. Extraction of the dried precipitate with water yielded very active ferment solutions of extremely low protein content. For the time being no satisfactory explanation was forthcoming concerning the nature of the influence of the fibrin ferment upon the "fibrin generators." There remained several possibilities.

The following years brought forth, in rapid succession, a number of publications by Schmidt (45-47) which concerned primarily the origin of the fibrin ferment, the participation of the formed elements in coagulation, and the effect of neutral salts. In 1876 Schmidt (49) published a summary of the results of his research. The latter will be dealt with in some detail since it constituted, in a sense, the conclusion of one period in the history of the study of coagulation just prior to the beginning of another.

In this discussion Schmidt defined the coagulation of fibrin as a transmutation process in which under the influence of a specific enzyme—fibrin ferment—and in the presence of a certain amount of neutral alkali salts, two originally soluble proteins are transformed into one insoluble product, i.e., fibrin. Circulating blood remains liquid because it does not contain fibrin ferment. This could be proved when blood was collected in alcohol as soon as it left the blood vessel. The ferment was first produced in fluids that possessed the ability to coagulate when they were removed from their natural milieu, and originated from the disintegration of the formed elements, especially of the white blood cells. A number of observations led Schmidt to look upon the white cells as the source of the ferment. These cells were the elements which largely disappeared and disintegrated during coagulation since there were materially fewer leucocytes in serum than in plasma, and only a few were incorporated into the clot. Furthermore, the slow coagulation in cooled

plastic substance" ("paraglobulin" of Bruecke [18]) a necessary substrate for coagulation. Hammarsten's investigations (117-123) were originally aimed at the production in a pure state of the individual substances essential for coagulation. These studies led to the discovery that coagulation was brought about by the fermentative (enzymatic) transformation of only one protein, fibrinogen. Hammarsten proved this by the production of fibrinogen and fibrin ferment free of "paraglobulin" which together could form a typical clot. The isolation of fibrinogen was accomplished by precipitation from magnesium sulfate plasma by an equal volume of saturated sodium chloride solution and repeated washing by dissolving in water and re-precipitating with sodium chloride. This process is still in use today with but minor modifications. The resulting colorless protein solution remained completely liquid. It no longer contained "paraglobulin." This could be demonstrated in many ways as for example by determination of the temperature of "heat-coagulation."* The protein solution, however, coagulated in a typical manner upon the addition of fibrin ferment.

Hammarsten's convincing evidence had put an important segment of Schmidt's theory in jeopardy. In rebuttal Schmidt (48) claimed that Hammarsten's fibrinogen solution in all likelihood contained "paraglobulin." Furthermore, Schmidt emphasized that there were exudates known to contain fibrinogen which did not coagulate upon the addition of fibrin ferment. But Hammarsten (118) and later Frédéricq (112-114) were able to repudiate Schmidt's

* The translators interpret this to mean a measurement of the temperature at which any specific protein may be denatured by heat and solidifies. This is a process quite different from physiological coagulation of plasma in which fibrinogen is converted to fibrin by thrombin (or, as Schmidt originally called it, fibrin ferment).

cipation of the "fibrinoplastic substance." Schmidt was aware of this and had already thought of the possibility of basing coagulation entirely upon the influence of the fibrin ferment on only one "fibrin generator," fibrinogen, and to forget the "fibrinoplastic substance" entirely. But he rejected this simple theory, chiefly because he occasionally found certain body fluids which did not clot upon the addition of fibrin ferment alone, but also needed the "fibrinoplastic substance." The latter substance could be obtained completely free of contamination with the ferment as for example from egg-white. Schmidt accepted these observations as definite proof for the absence of "fibrinoplastic substance" in body cavity fluids, but that on the other hand it must be present for coagulation to occur. Other findings, for example the dependence of the weight of the fibrin obtained upon the amount of "fibrinoplastic substance" present, seemed to indicate the importance of the latter.

Thus the theory of Alexander Schmidt seemed to form the skeleton of a proud structure. It apparently furnished a satisfactory explanation for the cause of coagulation as well as for the liquid state of the blood and thus to bring the discussion of coagulation to a conclusion—inasmuch as one can speak of a conclusion in connection with the problems of scientific research. But in the years that followed, while Schmidt and his students were extending his theory, many aspects of his thesis were challenged from numerous quarters.

As far as the writer has been able to ascertain, it was Hammarsten (117) who first took exception to Schmidt's teachings. He shared Schmidt's view concerning the ferment nature of coagulation and also recognized the importance of fibrinogen. However, he deviated from Schmidt's theory in that he did not consider the "fibrino-

This did not yet close the chain of proof with which Hammarsten opposed the theory of Schmidt. Hammarsten showed further that "paraglobulin" was found in plasma as well as in serum and in all exudates, and certainly in vastly larger amounts than had been hitherto assumed. Indeed, he thought it may well amount to more than 50% of the total protein content. In this respect Schmidt had apparently fallen victim to the inadequate methods which were available when he undertook the characterization of "paraglobulin" whereas Hammarsten had available the method of fractional precipitation with magnesium sulfate. The discovery of "paraglobulin" in those transudates which according to Schmidt did not coagulate upon the addition of fibrin ferment removed the final reason for considering "paraglobulin" a necessary factor for coagulation. Furthermore, the presence of a very large amount of "paraglobulin" in serum and plasma made it appear unlikely that it originated from extravascular disintegration of the leucocytes, as Schmidt had thought. It was more likely that "paraglobulin" already existed in circulating plasma and that its amount increased but little during coagulation.

Comparison of the analytically determined nitrogen values of fibrin with those of fibrinogen and "paraglobulin" yielded information incompatible with the hypothesis that fibrin resulted from the chemical union of the other two proteins. (It should be added that following the isolation of the fibrin ferment Schmidt no longer made such an assertion.)

Hammarsten also objected to another aspect of Schmidt's teachings (118). Schmidt thought that during the coagulation of fibrinogen there was an intermediate product which in turn was precipitated in the form of fibrin by neutral salts. Hammarsten showed that this was not entirely ten-

argument completely. There was then no longer any doubt that the "fibrinoplastic substance" did not play the role Schmidt had ascribed to it. It is unfortunate that only very much later did Schmidt convince himself of the untenability of his own theory, and then only in part (55).

Hammarsten's research marked by its clarity, logic, and care an important advance in the study of coagulation. It not only resulted in the conviction that "paraglobulin" was not essential for coagulation, but also clarified the reasons which had prompted Schmidt to arrive at his theory.

Hammarsten's observations showed first of all that it was possible to coagulate any body fluid containing fibrinogen by the addition of fibrin ferment (117, 118). Schmidt's negative findings were explained by the impotency of the ferment solutions which he used and the presence in transudates of anticoagulant substances, the effects of which were weakened by the presence of "paraglobulin." The influence of "paraglobulin" upon the amount of fibrin formed was similarly explained by Hammarsten by assuming that substances that tended to keep fibrin in solution, perhaps salts, were bound by the "paraglobulin." Other proteins such as crude casein and even salts such as $CaCl_2$ had analogous effects. Thus it was impossible to speak of a specific "paraglobulin" effect. Finally, in those instances in which a definite fibrinoplastic effect was observed in the "paraglobulin" precipitate, it was attributed to contamination with fibrin ferment, because the effect disappeared when "paraglobulin" was subjected to temperatures of 56° to 59°C. This procedure inactivated the fibrin ferment but did not "heat-coagulate" the "paraglobulin." Similarly it was possible by repeated precipitation of "paraglobulin" to actually free the latter of fibrin ferment and therefore of its apparent fibrinoplastic property.

ing to a variable extent the second phase of coagulation, the precipitation of fibrin, and that "paraglobulin" in turn neutralized the action of these substances. He based this belief on the observation that the amount of fibrin obtained by coagulation was always less than that of the available fibrinogen which could be quantitatively determined by salt precipitation. Often the amount was actually very much smaller. Following the coagulation of fibrinogen there remained in solution a substance which possessed the characteristics of a globulin, and coagulated at 64°C. This substance was present in blood serum and was called "fibrinoglobulin" by Hammarsten (122). A similar substance developed in the coagulation of fibrinogen by heat. Hammarsten did not think it likely that this substance pre-existed in the fibrinogen solution but thought that it originated from fibrin during coagulation. "Fibrinoglobulin" was either identical with soluble fibrin, or it was a reflection of the division of fibrinogen into a soluble substance and a less soluble one during the process of coagulation. (See page 90).

In addition to Hammarsten it was Frédéricq (112-114) to whom we are indebted for enlarging our knowledge concerning fibrinogen. His views were very much like those of Hammarsten. Like the latter, he denied, on the basis of his experimental findings that "paraglobulin" participates in coagulation, but recognized on the other hand the significance of the fibrin ferment. Important was his proof of the pre-existence in unaltered plasma of a protein, fibrinogen, which "heat-coagulated" at 56°C. Such plasma was obtained by double ligature of the jugular vein of a horse. When the procedure was done carefully and aseptically, there was no coagulation in the vein, as was previously shown by Hewson (26). The formed elements gradually sedimented and a native plasma of a high fibrin-

able since it was possible to bring about the coagulation of salt-free fibrinogen solutions of a low alkali content by the addition of salt-free ferment. Hammarsten, by the way, expressed himself very conservatively in this matter since he did not consider his findings necessarily conclusive.

Hammarsten was, however, in accord with Schmidt in assuming the formation of an intermediate product, a soluble fibrin, in the course of the fermentative transformation of fibrinogen to fibrin. From numerous observations Hammarsten concluded that there must be an alteration of fibrinogen by the ferment prior to the beginning of visible coagulation. It will suffice to mention but a few of these observations. Fibrinogen in hydrocoele fluid "heat-coagulated" at 60°C, and that in the blood at 56°C. When the ferment was added to the hydrocoele fluid and the mixture heated prior to clotting, "heat-coagulation" then occurred at 56°C. Similarly, ferment-free hydrocoele fluid remained clear during freezing and subsequent thawing, while the same fluid became turbid when it contained ferment. Pure fibrin solutions behaved similarly. A generous addition of salts to the coagulation mixture frequently stopped the precipitation of fibrin, even though the fibrinogen may have undergone an alteration. All these observations pointed with some degree of certainty to the formation of a soluble intermediate product in the coagulation of fibrin. This product incidentally was not identical with the soluble fibrin of Eichwald (107) which, according to Hammarsten, was nothing but a more or less denatured fibrinogen. Hammarsten distinguished sharply between actual clot formation, i.e., precipitation of fibrin, and transformation of fibrinogen into soluble fibrin, both of which he felt represented different phases of the coagulation process. He thought it was quite likely that certain substances such as salts or alkalis were capable of inhibit-

but always detectable amounts of fibrin ferment. Nevertheless, their findings did not remain unchallenged. Koehler (74) for one, found no fibrin ferment in the circulating blood, nor did Haycraft (200), who employed a different method which will be discussed later.

Koehler (74) and Edelberg (60) were more fortunate than Jakowicki in producing intravascular coagulation with fibrin ferment. Koehler used serum or defibrinated blood in his experiments. The injection of the latter immediately after defibrination but while still at body temperature, in many instances caused the death of an animal as the result of intravascular coagulation. Edelberg proved that in this case the fibrin ferment was the effective agent. He produced extensive intravascular thromboses with concentrated but virtually protein-free solutions of ferment prepared by Schmidt's method. The earlier, negative experiments were explained by the low potency of the ferment solutions used. This proved that fibrin ferment had essentially the same effect within the vessels as in extravascular blood.

It should be mentioned that Koehler (74) and Edelberg (60) observed in many instances an elevated temperature following injections of fibrin ferment which they ascribed to the latter. On the basis of these observations they ascribed to fibrin ferment a significant role in the origin of fever in general. This was the beginning of the "Ferment Intoxication" theory which met with considerable interest, although there was no lack of vigorous opposition, as for example on the part of Hammerschlag (199). Apparently this theory has now been abandoned.

Another group of studies by followers of Schmidt dealt with the origin of fibrin ferment from the formed elements of blood. Here should be mentioned chiefly the efforts of *Hoffmann (68), Heyl (67), Krueger (77-79), Harm-*

ogen content was present in the upper portion of the ligated segment.

Hammarsten's and Frédéricq's research shed much light upon the second phase of the coagulation process in explaining the role of fibrinogen and its transformation to fibrin. Meanwhile, the fibrin ferment and its origin became the object of the investigations of Alexander Schmidt and his students. During the eighties of the last century Schmidt's associates published numerous papers which both confirmed and extended results Schmidt himself had obtained earlier and which also in some instances compelled a modification of his original theory.

These papers can be arranged in several different categories. The experiments of Jakowicki (69), Koehler (74), Edelberg (60), and Birk (57) concerned the effects of fibrin ferment in the living body and the ferment content of circulating blood. Naunyn (383), a pioneer in the production of intravascular coagulation by the injection of proteins, doubted the significance of fibrin ferment since in his hands it did not bring about coagulation when injected into the circulation. Naunyn and Francken (365), then adherents of Alexander Schmidt's original theory, attributed their observation that the injection of laked blood produced intravascular coagulation to the presence of "fibrinoplastic substance." Actually, neither Schmidt nor Jakowicki succeeded in producing intravascular coagulation by the injection of fibrin ferment. They believed in the ability of the body to inactivate a certain amount of this substance. This seemed all the more probable since it was assumed that there was normally some development of small amounts of fibrin ferment in circulating blood. In order to support this belief, Jakowicki and Birk (57) collected blood directly into alcohol and determined the ferment content by Schmidt's method. They found variable

by Schmidt's method. If the tissues contained active fibrin ferment, they would also have to bring about coagulation in any fluid containing fibrinogen. As previously discussed, this was not the case at all. It had to be assumed then that blood plasma possessed the ability to activate the fibrin ferment, but that certain transudates did not. It will be seen that Rauschenbach's ideas, developed in a very readable and careful study, came very close to modern beliefs concerning the origin of fibrin ferment and differed from them only in terminology. Rauschenbach also defended Schmidt's ideas concerning the disintegration of leucocytes in shed blood and believed that such had already occurred to some extent in the circulation.

This study was supplemented by the observations of Grohmann (62) and Groth (63). Grohmann showed that not only animal protoplasm, but likewise vegetable protoplasm, especially bacteria, brought about the production of fibrin ferment when mixed with plasma. Groth investigated the effect of cells in the circulating blood which had been previously studied by Wooldridge (292) although from a different point of view. Intravascular coagulation was much more readily produced by the injection of cells than by that of fibrin ferment. The injected cells disappeared from the blood very quickly. Indeed, considerable leucopenia resulted, and the blood, while still liquid, more or less lost its propensity for spontaneous coagulation, as well as its ability to break down protoplasm with the simultaneous development of fibrin ferment.

Von Samson (85) and Nauck (81) performed experiments designed to isolate the inactive precursor of fibrin ferment, the existence of which had become a probability through the efforts of Rauschenbach. They claimed to have found substances such as various relatively simple nitrogen-containing ones, especially amino acids, which were able to produce the same effect as fibrin ferment.

sen (65), and Berg (56). All of these workers attempted to prove Schmidt's theory concerning the role of leucocytic disintegration in coagulation, largely by way of comparative leucocyte counts in blood and in serum following coagulation. The most important feature of these studies was the discovery that the number of leucocytes, particularly the polymorphonuclear cells, always decreased considerably during coagulation, sometimes by as much as 70 per cent. It was considered unlikely that stirring the blood led to the trapping and subsequent removal of the leucocytes by the fibrin. This impression was based on Heyl's observation that only an infinitesimal fraction of the red cells (approximately 1 per cent) disappeared during coagulation, probably by incorporation into the fibrin network (67).

These investigations seemed to confirm Schmidt's beliefs concerning the importance of the leucocytes in the production of fibrin ferment. On the other hand, the studies of Rauschenbach (82), Grubert (64), Groth (63), and Grohmann (62) demonstrated the need for an extension of Schmidt's theory. They showed that not only leucocytes but every type of protoplasm could be broken down by blood plasma and therefore in turn could yield fibrin ferment. This was most thoroughly demonstrated by Rauschenbach, who investigated the influence of various tissue cells upon blood plasma. All of the cells studied accelerated greatly the coagulation of salt-plasma, cooled plasma, and some exudates while they remained oddly ineffectual when added to certain other transudates and exudates. Rauschenbach postulated the existence in all tissues (especially in multi-nucleated cells such as leucocytes and spermatozoa) of a precursor of fibrin ferment which he named "protozyme." On the other hand, the presence of active fibrin ferment was not assumed since tissues and blood leucocytes did not yield fibrin ferment

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They used bile salts as a reagent, i.e., a plasma which had been prevented from coagulating by the addition of 1 to 2% of bile salts. Their experiments concerned solely the acceleration of coagulation and not its initiation. For this reason they proved little and their results were subsequently interpreted differently by Schmidt. Nauck and von Samson, by the way, found that the accelerating effect of laked blood which Schmidt had attributed to hemoglobin had nothing to do with the latter, but was a function of the stroma of the erythrocytes.

The studies of the Dorpat school relative to the origin of fibrinogen and similar problems were of lesser importance than the investigations so far discussed. One should mention the findings of Semmer (87) who thought he proved that a fibrin-like substance could be extracted from the nucleated red cells of frogs and birds. Kollmann (75) later took the position that all cells of the body were capable of contributing to the formation of fibrin by releasing proteins into the blood which then passed through various intermediate stages and finally ended up as fibrinogen or fibrin.

It was only natural that the work of other scientists, especially of men of his own school, influenced Schmidt's ideas. This in addition to his own continued research led him to extend his theory and to modify it in some respects. In his last two publications, Schmidt presented the sum total of his past experiences and of his more recent observations in the form of his third coagulation theory (54, 55). This is perhaps not entirely the correct term. Schmidt himself denied vigorously that he ever formulated another coagulation theory after the discovery of fibrin ferment. His first summary of 1876 presented a well-rounded picture of the coagulation process which, although it could have been supplemented at some points, represented by and large a unified concept. However, in many passages

his later work betrayed a spirit of resignation, a feeling that he would not be able to bequeath to later generations his life's work, the coagulation theory, in the definitive and final form for which he had hoped.

The ideas which Schmidt put into these writings differed from his earlier beliefs insofar as his theory concerning the origin of fibrin ferment had been greatly modified, while the importance of the "fibrinoplastic substance" (the "paraglobulin") had been de-emphasized. Now Schmidt concerned himself more with the part played by the "fibrinoplastic substance" in the formation of fibrinogen, rather than of fibrin.

In the first chapter of his book, *The Theory of Blood*, Schmidt stressed the essentially cellular nature of coagulation. According to this, in the last analysis not only the fibrin ferment but also the coagulation substrate, fibrinogen, were derived from cell protoplasm. At least two phases of coagulation had to be differentiated: the origin of fibrin ferment from its inactive precursor and the effect *of the ferment upon fibrinogen (and according to Schmidt* also upon the "fibrinoplastic substance"). The ferment effect led to the formation of soluble fibrin which neutral salts then precipitated in an insoluble form as the fibrin clot.

The first phase of coagulation, the origin of fibrin ferment, was considered to be an exceedingly complex process which could not be sufficiently explained by the simple disintegration of white cells. Schmidt did not think that the white cells contained fibrin ferment since they were not effective on typical "proplastic"* fluids (e.g., certain transudates, body fluids of horses, and most hydrocoele fluids). The leucocytes may well contain, as Rauschenbach (82) thought, an inactive precursor which is converted into

* Those serous cavity fluids which contained fibrinogen but did not clot spontaneously. See page 15.

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hanced by the addition of "zymoplastic agent," so that the serum now was far more potent than in its original state. Its clot-promoting effect upon fibrinogen solutions increased 20 to 30-fold. Schmidt attempted to explain the situation as follows: In normal coagulation only a small portion of the prothrombin in the plasma was activated by the "zymoplastic agent" released from the leucocytes. A considerable part, however, was found in serum in the much more resistent form of prothrombin. This was due to the fact that certain anticoagulants quickly neutralized the activity of the "zymoplastic agent." Thus there existed *in serum a state of balance between anticoagulants and* procoagulants.

Whenever this balance was upset by the addition of "zymoplastic agents," naturally more prothrombin was converted to thrombin. A temporary increase in the alkalinity of the serum had a similar effect. Here, too, the repeated formation of extraordinarily large amounts of thrombin could be frequently observed. Schmidt believed that alkali was not effective per se but only increased the activity of the "zymoplastic agents" already present in the serum.

Except for its physiological impotency and its greater resistance to certain chemical influences, prothrombin was considered to be not significantly different from thrombin. It was no more heat-resistant, could not be dialysed, and was precipitated with the globulins.

In addition to the "zymoplastic agent" cells were thought to contain another substance of importance for coagulation, "cytoglobin." This substance inhibited coagulation and was isolated by drying the cells with alcohol and extracting the residue with water. "Cytoglobin" was considered a substance similar to albumin containing a large amount of phosphorus and possessing definite anticoagulant activity. As little as 1 per cent added to blood

fibrin ferment by the effect of blood plasma. In contrast to Rauschenbach, however, Schmidt thought that the inactive precursor was located in the plasma, while he theorized that the substances which brought about the activation of the precursor were found in the blood cells or in protoplasm in general. Thereafter, Schmidt referred to the fibrin ferment as "thrombin,"* and to the inactive precursor as "prothrombin." These terms have come to be commonly used, at least in Germany. The active cell substances were called "zymoplastic agents."† As they entered the plasma they supposedly activated prothrombin. They were considered "not the mothers, but the producers" of thrombin. The "zymoplastic agent" was not considered to be chemically homogeneous but to consist of several agents which could be extracted from the cells by means of alcohol. They were heat-resistant which proved that they were of a different nature than thrombin. Concentration of the alcoholic cell extracts yielded a yellow, fatty powder. These substances had no effect on "proplastic" fluids while they greatly accelerated or initiated coagulation in salt-plasma and cooled plasma. The clot-promoting effect of these cells which was demonstrated by the experiments of Rauschenbach (82) and Groth (63) was attributed to the presence of these alcohol-soluble substances. The effect of the "zymoplastic agent" upon blood serum was a most interesting phenomenon. Blood serum contained a certain amount of active thrombin. When serum, especially horse serum, was exposed to air, the amount of thrombin rapidly decreased to the extent that it quickly became totally or largely inactive. The thrombic activity of this serum could, however, be greatly en-

* *Translators' note:* Although Morawitz subsequently used the term "fibrin ferment" in the original article, the translators will use only the modern term, "thrombin."

† *Translators' note:* In modern terminology this would probably correspond to "thromboplastin" or "thrombokinase."

bin. In support of this theory Schmidt pointed out the striking increase in the weight of the fibrin obtained upon the addition of "cytoglobin" as well as "paraglobulin."

From this it was concluded that coagulation appeared in the last analysis to be an exclusively cellular phenomenon which could be graphically represented as shown in Figure I.

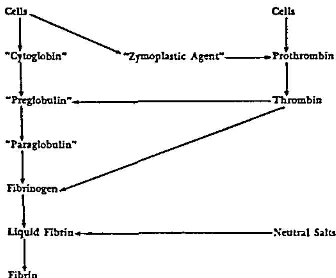


FIG. I.

At this point we may interject a few critical remarks concerning this last coagulation theory of Schmidt. This report has granted considerable space to the discussion of the origin of thrombin since Schmidt's findings did not excite much comment during the period that followed. Only in more recent days have they been confirmed by further research and given their proper recognition.

The same cannot be said about "cytoglobin" and the role it was thought to play in coagulation, especially in

completely inhibited the coagulation process. Von Rennenkampff (83) showed that it had the same effect when injected intravascularly. The action of "cytoglobin" on thrombin was not very great since even large quantities could not prevent the coagulation of fibrinogen solutions by thrombin. But "cytoglobin" inhibited the formation of thrombin, i.e., its production from prothrombin. Therefore, "cytoglobin" was thought to exert its inhibitory action on the "zymoplastic agent." Schmidt was inclined to ascribe to "cytoglobin" an important role in the preservation of the liquid state of the blood. However, he was not able to furnish chemical proof of the existence of "cytoglobin" in blood plasma. Nevertheless, he did not consider it risky to assume that the anticoagulant portion of the molecule of "cytoglobin" might exist in the blood since many observations convinced him that serum contained anticoagulants which were not salts but which could be removed by dialysis.

On this basis Schmidt explained the fluid state of blood as follows: Inactive prothrombin existed in the plasma of circulating blood. On a modest scale constant disintegration of formed elements in the blood released the "zymoplastic agents" into the plasma. These would result in the formation of thrombin if they were not checked by the presence of an anticoagulant. The disintegration of the leucocytes in *shed* blood suddenly released large amounts of "zymoplastic agents" into the plasma. The balance was upset. Thrombin was then formed. However, during the formation of thrombin only part of the available prothrombin was converted (see above).

Schmidt ascribed a second function to "cytoglobin." He considered it the precursor of fibrinogen into which it developed via several intermediate stages which were called "preglobulin" and "paraglobulin." These extensive transformations were supposed to be brought about by throm-

Chapter III

THE ROLE OF CALCIUM SALTS IN COAGULATION

ALEXANDER SCHMIDT maintained that neutral salts were necessary for coagulation, considering it their function to transform soluble fibrin into fibrin. He thought that all soluble salts of alkalis (including earth alkalis) influenced the process in the same manner, and that coagulation depended upon the presence of salts in general rather than upon any specific salt. This, naturally, did not exclude quantitative differences in the activity of various salts. Indeed, in Hammarsten's first study of 1875 he had already referred to the finding that $CaCl_2$ had a particularly striking effect upon the speed of coagulation as well as upon the amount of fibrin formed (117). Hammarsten maintained that in this sense $CaCl_2$ could be called a "fibrinoplastic substance" just as well as "paraglobulin" could. At first he thought that $CaCl_2$ played the same role in coagulation as rennin did in the fermentation of milk. However, he could not substantiate this theory since thrombin could transform fibrinogen into fibrin in the absence of calcium salts. Hammarsten subsequently did not discuss the specific role of calcium salts.

A few years later Green (164) discovered that the clotting of salt plasma or of any other slowly coagulating plasma could be appreciably accelerated by the addition of a solution of gypsum ($CaSO_4 \cdot H_2O$), whereas thrombin-free serous fluids (e.g., hydrocoele fluids) could not be clotted by gypsum. Therefore, he thought that calcium

relation to fibrinogen. As far as I know there has been no further investigation of "cytoglobin." However, from Schmidt's data one can certainly conclude that "cytoglobin" is nothing but more or less contaminated nucleoprotein. There is no doubt that it inhibits coagulation. On the other hand, it is doubtful that this substance plays any role in normal coagulation, particularly since "cytoglobin" was anticoagulant only in a very strong concentration. But Schmidt deserves credit for pointing out that there had to be a substance in the circulating blood which could prevent coagulation, and for presenting some evidence for its existence.

Schmidt's theory concerning the transformation of "cytoglobin" into fibrinogen through the stage of "paraglobulin" raises even stronger doubts. Apparently, he had been led to speculate with insufficient foundation in the available data. In this, he was obviously guided by the desire to re-affirm in a new form his earlier, vigorously defended teachings concerning the participation of the "paraglobulin," i.e., the "fibrinoplastic substance," in coagulation. In its earlier version his theory had already been invalidated by the investigations of Hammarsten.

This partially speculative aspect of Schmidt's theory may explain in part why his ideas failed to gain wider recognition and why in particular his studies relative to the origin of thrombin have not been systematically tested. The coagulation theory which prevailed around 1890 resulted largely from the careful investigations of Hammarsten and can be formulated approximately as follows: *Coagulation is brought about by the fermentative transformation of but one protein, fibrinogen, into fibrin. The ferment exists only in shed blood and originates in all probability from the white blood cells when they disintegrate in shed blood.*

lar wall and established contact with foreign bodies, they were damaged by adhesion. The phosphates then escaped and a precipitate of tri-calcium phosphate formed in the plasma. This in turn precipitated fibrin, perhaps in a purely mechanical fashion. This theory which in essence constituted a return to the older physical theories of coagulation was supported only by scanty and by no means incontrovertible evidence.

It is doubtlessly true that adhesion, i.e., contact with foreign bodies, plays an important part in coagulation. This fact which had already been recognized by Bruecke was illustrated by the following appealing experiment of Freund: *When blood was collected in oil or vaseline, it* remained fluid for several hours, even when agitated with a greased glass rod. However, it coagulated instantly when put in contact with a foreign body not covered with oil or vaseline (417). Thus the importance of adhesion (i.e., contact with a wettable foreign surface) to coagulation was rendered quite apparent. Freund's experiment was later confirmed by Haycraft in support of the latter's belief that thrombin was absent in circulating blood (200). More recently Bordet and Gengou (185) and others have successfully used the same method to obtain a comparatively unaltered plasma. In these experiments blood was collected and centrifuged in paraffined containers. The objections of Strauch (88) to this portion of Freund's theory are not valid.

The second portion of Freund's theory was unfortunately less well founded and was actually based on no more than the following finding: The addition of calcium phosphate considerably accelerated the coagulation of some fluids which coagulated spontaneously anyway, and the weight of the fibrin obtained was thus increased.

The observations of Latschenberger (168, 169), Strauch

salts potentiated the thrombin effect but could not substitute for it. Green suggested the possibilities that calcium may activate a precursor of thrombin or may combine with fibrinogen to form fibrin under the influence of thrombin. His observations, however, could not prove one or the other of his theories. In the first place he did not succeed in isolating a precursor of thrombin from blood collected in alcohol and precipitated by the method of Schmidt. In the second place he found that the amount of fibrin obtained was not directly influenced by the amount of calcium used. Even though Green's work did not provide an explanation for the effect of calcium, his observations made it probable that calcium salts were necessary for coagulation even though they operated in an unknown manner. Moreover, Ringer and Sainsbury (171) soon pointed out that not only gypsum, but all other soluble calcium salts (and to a certain extent strontium and barium salts) in low concentration augmented coagulation considerably.

Specific theories concerning the role of calcium salts were first noted in the studies of Freund (161, 162), who took the earlier findings of Bruecke (17) as his point of departure. Based on personal observations, Freund developed a completely new and surprising theory of coagulation. Bruecke had demonstrated that the ash of fibrin contains calcium phosphate, a finding which stimulated Freund to investigate the importance of that particular salt. Freund then concluded that calcium phosphate played a very important role in coagulation. According to this, soluble calcium salts were found in the circulating plasma, while only the *blood cells contained phosphates*, especially alkali phosphates. As long as the cells were intact, the phosphates could not enter the plasma. As soon as the cells were removed from the influence of the vascu-

the same effect but not barium or magnesium salts. How could these surprising and interesting findings be explained? With what phase of coagulation were calcium salts concerned? Were they needed for the production of thrombin, or did they participate in the conversion of fibrinogen to fibrin? Finally, there existed the possibility that they could take part in both phases of coagulation.

These questions which had already been raised by Green (164), were answered in many ways by the discoveries of Arthus and Pagès (154). It can truthfully be said that these and other astute observers did not omit any possible explanation.

Arthus and Pagès favored the view that calcium salts were needed for the conversion of fibrinogen to fibrin but not for the production of thrombin from its precursor. They felt that oxalate plasma was not lacking in thrombin, but that the latter could not act upon fibrinogen in the absence of calcium salts. Accordingly, the addition of serum (which contains thrombin), or of Schmidt's thrombin preparation, would not produce coagulation in oxalate plasma. The latter, on the other hand, could coagulate fluids containing fibrinogen because of its thrombin content. Arthus and Pagès also demonstrated that under certain experimental conditions the weight of the fibrin obtained definitely depended upon the amount of the calcium salts present. These findings led Arthus and Pagès to conclude that calcium salts combined with fibrinogen under the *influence of thrombin. In a later publication* (155) Arthus stressed numerous and far-reaching analogies between the coagulation of blood and the fermentation of casein by rennin. The chief difference was that rennin converted casein into paracasein in the absence of calcium salts, while he thought that thrombin could be effective only in the presence of calcium. In this connection Arthus

(88), and Arthus (153) soon demonstrated that Freund's theory of coagulation was inconsistent with many findings. Strauch showed that certain fluids containing fibrinogen (such as transudates which did not clot spontaneously) also did not coagulate upon the addition of calcium phosphate, i.e., the salt could not substitute for thrombin. Similar objections resulted from later experiments which showed that Freund's theory was unsuited to explain the role of the calcium salts or the process of coagulation. Pekelharing (170) demonstrated subsequently that injection of sodium diphosphate into the blood stream did not bring about intravascular coagulation. This observation deprived Freund's theory of its last measure of support.

Considerable progress toward recognizing the importance of calcium salts in coagulation was made by the studies of Arthus (155, 156) and Arthus and Pagès (154). It was the distinction of these two investigators to have been the first to state emphatically that calcium salts were in some way absolutely necessary for coagulation. They showed that when shed blood was collected in an alkali oxalate solution in a proportion of one gram of oxalate per liter of blood, the latter remained fluid and yielded a plasma which showed no turbidity after the addition of more oxalate. Therefore, this plasma no longer contained more precipitable calcium salts. Other calcium-precipitating substances had the same effect, especially sodium fluoride which prevented coagulation in a concentration of 0.15 to 0.3 per cent. Stronger concentrations of alkali soaps had a similar effect.

Proof that the precipitation of the calcium salts was responsible for the incoagulability of the blood was furnished by the subsequent addition of a slight excess of calcium salts which quickly brought about the coagulation of the oxalate plasma. The addition of strontium salts had

magnesium sulfate plasma. It was precipitated along with the globulins. The globulin precipitate which contained the precursor had no thrombin activity. It became active as soon as it was mixed with a solution of calcium chloride. Thrombin was then produced. Once produced, thrombin could not again be inactivated by the addition of oxalate. It could coagulate fluids containing fibrinogen and even oxalate plasma in the absence of calcium salts. The negative findings of Arthus and Pagès were explained by the impotency of their thrombin solutions and the fairly marked anticoagulant effect of oxalate.

Pekelharing considered thrombin to be a calcium compound of its precursor and to contain calcium in a form which could not be precipitated by oxalate. He found that the ash of thrombin contained much calcium. He not only considered calcium of importance for the first but also for the second phase of coagulation. In this instance he compromised with the interpretation of Arthus. Fibrin was thought to be a calcium-protein compound, and the function of thrombin that of absorbing calcium from plasma and transferring it to fibrinogen. Thrombin, or rather its precursor, was thus considered nothing but an agent for calcium-transport. Pekelharing supported this hypothesis by comparing the calcium content of heat-precipitated fibrinogen to that of fibrin. The latter was always found to be materially greater. Therefore, while Arthus considered calcium salts necessary only for the second phase of coagulation, Pekelharing stressed their importance in both phases.

The second part of Pekelharing's theory is difficult to understand as Hammarsten later emphasized (165). If thrombin absorbed calcium from plasma and transferred it to fibrinogen, and if fibrin was a calcium compound of fibrinogen, then the formation of fibrin should stop as

spoke of coagulation as a "caseinification" of blood, considering fibrin as a "cheese," or the calcium salt of fibrinogen.

While the observations of Arthus and Pagès were soon confirmed, their theory did not meet with general approval. On the basis of numerous, thorough investigations, Pekelharing arrived at a vastly different idea concerning the significance of soluble calcium salts (170, 210-213). He attempted first of all to prove that oxalate plasma did not contain active thrombin as Arthus and Pagès had assumed. *He found that oxalate plasma was not capable of producing* coagulation in magnesium sulfate plasma (as Schmidt had thought) or in fibrinogen solutions. Since Arthus, on the other hand, assumed that thrombin was effective only in the presence of calcium salts, the experiment with the fibrinogen solution was not convincing. However, the experiment demonstrating that magnesium sulfate plasma, which contains calcium, always clotted upon the addition of thrombin was convincing. These observations suggested the possibility that oxalate plasma might contain a precursor of thrombin which was converted to active thrombin by calcium salts. Green had already considered this possibility but subsequently discarded it. Indeed, the existence of prothrombin which could be activated by calcium salts can be demonstrated. When a fibrinogen solution was obtained from oxalate plasma by salt precipitation, the solution at first still coagulated upon the addition of calcium salts, but after repeated precipitation it lost this property. Apparently, a substance had been removed which in combination with calcium salts yielded thrombin, but was not actually thrombin, since, as was mentioned before, active thrombin does not exist in oxalate plasma.

This substance, the precursor of thrombin, was obtained in comparatively large amounts from oxalate plasma or

tate formed which could be dissolved in sodium carbonate but would re-precipitate when calcium salts were added. This latter precipitate was considered to be fibrin. Therefore, Lilienfeld concluded that fibrin is a calcium salt of "thrombosin," and called it, according to Arthus' nomenclature, a "thrombosin cheese." (See page 49).

Lilienfeld believed that while the acidic "leuconuclein" brought about coagulation, the alkaline "histone" possessed strong anticoagulant properties both intra- and extravascularly. Plasma containing "histone" did not coagulate upon the addition of thrombin but did when "leuconuclein" was added. (See page 108).

Both molecular complexes, the procoagulant as well as the anticoagulant, were thus considered to be combined in one large molecule as "nucleohistone." Both the liquid state of blood and coagulation were explained by an antagonism between these two factors. The intravascular injection of "nucleohistone" consequently produced at times intravascular coagulation and at other times an incoagulable state of the blood. At times both phenomena occurred simultaneously, i.e., some thromboses appeared while the rest of the blood remained incoagulable.

Lilienfeld's theory concerning the role of calcium was quickly refuted (see below). It should be noted that the results he obtained in experiments with "leuconuclein" were by no means incompatible with the thrombin theory of coagulation as will be shown later. The same can be said for the anticoagulant, "histone," as was said for Schmidt's "cytoglobin" with which it was apparently identical. I have on many occasions convinced myself experimentally that the inhibitory effect is undoubtedly present, but there is no proof that "histone" normally participates in coagulation. This could be easily demonstrated since only a fairly strong concentration of "histone" interfered

soon as there were no more calcium salts left in the plasma. However, this was not the case, as Pekelharing himself demonstrated in opposition to the views of Arthus and Pagès. Active thrombin was effective in the absence of calcium salts which had been previously precipitated by oxalate. Consequently, the second part of Pekelharing's theory was untenable.

Lilienfeld (428-433) had a completely different concept of the role of the soluble calcium salts. On the basis of numerous chemical and anatomical experiments Lilienfeld developed a theory of coagulation which not only ascribed to calcium salts a unique role, but which in many other respects was at variance with prevailing opinions. In agreement with Freund, Lilienfeld, while admitting the existence of thrombin, denied its significance in coagulation, and attempted to show that coagulation depended in a critical manner upon the nucleoproteins found in the nuclei of the leucocytes and in blood platelets. He isolated a "nucleohistone" by precipitating it from aqueous cellular extracts with acetic acid. This "nucleohistone" was thought to play an important role in coagulation. When treated with mineral acids this substance was supposed to split into "leuconuclein" which consisted of nucleic acid and protein, and an albumose-like substance, "histone." Like thrombin, the "leuconuclein" was thought to possess the ability to initiate coagulation in all fluids containing fibrinogen in the presence of calcium. Lilienfeld felt that this property depended on the acid nature of the "leuconuclein." Like the latter substance, nucleic acid, and even simple organic acids, were considered capable of splitting fibrinogen into a protein which could not be dissolved by acid, the so-called "thrombosin," and an albumose. When a fibrinogen solution prepared according to Hammarsten's method was mixed with acetic or nucleic acid, a precipi-

coagulate upon the addition of thrombin was also incorrect, because it was quite easy to clot fibrinogen solutions (containing no calcium and little oxalate), "proplastic"* fluids, etc., with thrombin.

The preceding observations of Schmidt indicated that the theory of Arthus and Pekelharing that fibrin was a calcium compound of fibrinogen could not be correct. There was further proof of this in the demonstration that the calcium content of fibrin was not inherent in the latter but due to contaminants, primarily "zymoplastic agents," which could be removed by extraction with alcohol and ether.

Thus calcium salts were not considered necessary for coagulation, but merely to favor the formation of thrombin from its inactive precursor and the precipitation of fibrin. Schmidt thought *that calcium salts did not have a specific* role since they could be replaced by other salts. Whenever the accelerating effect of calcium salts in an artificial coagulation system containing fibrinogen was particularly marked, *Schmidt believed it was simply due to the fact* that fibrinogen solutions prepared according to the method of Hammarsten contained an excess of sodium chloride.

Arthus (156) undertook to refute Alexander Schmidt's objections to his theory of the specific significance of the calcium salts. He did not succeed completely. He demonstrated, to be sure, that oxalate per se did not possess the *strong anticoagulant effect which Schmidt ascribed to it.* But he disregarded Schmidt's basic experiment which showed that it was possible to coagulate calcium-free fibrinogen solutions with calcium-free thrombin. Arthus believed *that oxalate plasma clotted during dialysis be*cause of the calcium content of the sodium chloride solu-

* See footnote page 29.

with coagulation, and therefore, "histone" would have to be present in relatively large amounts. To date there is no proof that it is. As far as I know, neither "histone plasma" nor "cytoglobin plasma" have since been the object of detailed investigation. We are, therefore, in no position to say what the anticoagulant effect of "histone" is based on or which of the coagulation factors its acts upon.

Various investigators, as we have seen, held widely divergent views concerning the role of the calcium salts. There was, however, considerable agreement that those calcium salts which can be precipitated by oxalate were necessary for coagulation and that they had a specific role. Schmidt, on the other hand, remained completely aloof from these new ideas. In the third chapter of his last monograph (55) he opposed the ideas of Arthus and of Pekelharing. According to Schmidt, oxalate plasma did not remain fluid because it lacked calcium salts, but because it contained an excess of oxalate. Oxalate prevented coagulation in a much lower concentration than the other neutral salts, particularly by inhibiting the production of thrombin from prothrombin. When excess oxalate or fluoride, another anticoagulant, was removed from the plasma (which was accomplished most simply by dialysis against a dilute sodium chloride solution), coagulation occurred in the dialysate without the participation of calcium salts.

The strong anticoagulant effect was not restricted to oxalate and other calcium-precipitating salts, but was also found in alkali citrates which did not precipitate calcium. As little as 0.3 to 0.5 per cent potassium citrate sufficed to keep blood fluid, and yet the plasma still contained calcium salts in abundance. This was shown by the addition of oxalate which caused a distinct turbidity in the citrate plasma. Arthus' observation that oxalate plasma did not

the centrifuged plasma was by no means free of calcium. It still apparently contained readily demonstrable amounts of calcium which could not be precipitated by oxalic acid and must therefore have been more firmly bound, probably by organic compounds. One could not therefore speak simply of calcium, but only of that portion which could be precipitated by oxalate. Only the latter was considered of interest in the investigation of coagulation.

When clear oxalate plasma was allowed to stand for a time at low temperature, a granular sediment gradually formed. After this was removed, the plasma progressively lost its ability to clot upon the addition of calcium salts. Coagulation then occurred only slowly or not at all. Therefore, a substance must have been removed in the precipitate which augmented or initiated coagulation in the presence of calcium salts.

After the removal of this substance it was possible to obtain a fibrinogen solution from oxalate plasma according to the previously described method of Hammarsten (by precipitation with calcium-free sodium chloride and repeated purification by dissolving and re-precipitating). This fibrinogen solution did not clot upon the addition of $CaCl_2$ but did after mixture with a solution of thrombin which had been decalcified by oxalate (e.g., decalcified blood serum). Contrary to the observations of Arthus, oxalate plasma, like a solution of fibrinogen, could be clotted by decalcified thrombin, but this was somewhat more difficult because of the fairly strong anticoagulant effect of the excess oxalate.

Thus Hammarsten proved that those calcium salts which could be precipitated by oxalate were not required for the second phase of coagulation. Thrombin could convert fibrinogen into a typical fibrin clot in the absence of such salts. Lilienfeld and Arthus were in error in this respect;

tion used as the perfusate, but he offered no proof of this. The anticoagulant effect of citrate according to Arthus was due to the fact that citrate modified calcium without precipitating it, so that calcium was incapable of taking part in coagulation. Recent investigations have supported Arthus' viewpoint. Sabbatani (172-174) proved that calcium ions are needed in coagulation and that sodium citrate (and strong concentrations of other salts) retarded or abolished the ionization of calcium salts.

These considerations showed that the conclusions of different investigators concerning the role of calcium salts were widely divergent and frequently contradictory, and that even the results of their experiments were often greatly at variance with one another. According to Arthus and Lilienfeld calcium salts were absolutely necessary for the second phase of coagulation. Fibrin, according to these workers, was a calcium compound of fibrinogen or of "thrombosin." Pekelharing assumed the need for calcium salts in both phases of coagulation. He considered thrombin to be a calcium compound of its precursor and to possess the ability of transferring calcium to fibrin. Fibrin was thus also considered to be a calcium compound. Finally, Schmidt categorically denied that calcium salts had an effect upon any phase of coagulation.

It is to Hammarsten's great credit that he clarified these seeming contradictions and recognized the true role played by the calcium salts in coagulation (165, 166). He posed the following questions: (1) Was the presence of calcium salts a *sine qua non* for the action of thrombin upon fibrinogen? (2) Were calcium salts at all necessary in coagulation?

First of all he confirmed the important observation of Arthus that blood collected in an oxalate solution of sufficient concentration remained fluid. He also showed that

disproved. Hammarsten (165, 166) showed by means of a long series of calcium analyses that (1) the calcium content of fibrin need not be greater than that of heat-precipitated fibrinogen, and (2) that it was possible to obtain fibrin containing only barely detectable traces of calcium (0.007 to 0.0095 per cent). If one still believed in the calcium-salt nature of fibrin, Hammarsten showed that it would be necessary to assume a molecular weight for fibrin so great in magnitude as to be quite improbable.

Hammarsten furthermore thoroughly refuted that part of Lilienfeld's theory which concerned the role of the calcium salts. He showed that "thrombosin" was nothing other than fibrinogen precipitated by calcium salts in a solution of low salt content. Lilienfeld's fibrin was thus not a genuine fibrin. Cramer* and Schaefer (175) concurred with Hammarsten's opinion.

These studies of Hammarsten's highlighted another chapter in the theory of coagulation. All subsequent observers confirmed his observation that oxalate-precipitable calcium salts participate specifically only in the first phase of coagulation, i.e., in the formation of thrombin. Furthermore, Arthus (1) and Pekelharing (213) modified their theories in accordance with Hammarsten's opinion which so far remains unchallenged. Recent studies concerning mainly the anticoagulant effect of larger concentrations of calcium salts are of little interest for the theory of coagulation and should be mentioned only in passing.

Hammarsten was cautious enough to omit in his publications specific statements concerning the mechanism by which he thought calcium salts brought about the production of thrombin. This problem, which was closely allied to studies concerning the chemical nature of thrombin, subsequently became the subject of a series of publica-

* *Over de Zoogenaande Thrombosin.* Utrecht, Diss. 1896.

Pekelharing's and Schmidt's ideas regarding this point were correct.

On the other hand, Hammarsten showed that Schmidt was in error in considering calcium salts as altogether unnecessary for coagulation. Contrary to Schmidt's findings, he found it impossible to bring about the coagulation of oxalate plasma by dialysis against calcium-free sodium chloride solution. Schmidt's finding could be explained by the fact that his oxalate plasma in all likelihood already contained traces of thrombin which became effective only after the removal of the oxalate excess which had impeded coagulation. Carefully collected oxalate plasma did not contain thrombin but a substance which in conjunction with calcium salts furnished active thrombin. This point had already been stressed by Pekelharing. This substance was also found in the granular precipitate which formed when oxalate plasma was cooled to 0°C. In the absence of calcium salts this precipitate was completely inactive on a fibrinogen solution. It acquired thrombin activity after treatment with calcium salts. It thus seemed to contain prothrombin which was somehow activated by calcium salts.

Therefore Hammarsten proved that calcium salts were absolutely necessary in coagulation and played a very specific role. Calcium salts could not (in contrast to what occurs in rennet fermentation) be replaced by other salts with the exception of those of strontium. Those calcium salts which could be precipitated by oxalate were needed only in the first phase of coagulation. While they were not needed in the second phase (the effect of thrombin upon fibrinogen), their presence greatly augmented this reaction.

This made the possibility that fibrin was a calcium-salt compound most unlikely. This idea was also experimentally

Chapter IV

THROMBIN AND THE CLOT-PROMOTING SUBSTANCES IN TISSUES

It is only natural that the discovery and general recognition of thrombin should have stimulated investigations concerning its chemical nature and its distribution in the body. Alexander Schmidt apparently did not think that thrombin was a protein but did not consider the question in detail. On the other hand, English investigators attempted quite early to isolate thrombin in a pure state but did not obtain consistent results. The chemical nature of thrombin remains to this day as poorly understood as the chemical nature of all other enzymes. Older studies generally favored the belief that thrombin was protein in nature. It is, however, quite doubtful that the thrombin preparation obtained from blood serum by Schmidt's method was protein in nature. That particular method as has been emphasized by Lea and Green (205) yielded solutions of an extraordinarily low protein content which, however, had considerable thrombin activity. Thus far the production of thrombin solutions completely free of protein has not been possible, and therefore definitive proof against the protein nature of thrombin has not been obtained. Although the reaction between thrombin and alcohol has often been quoted as evidence against the protein nature of this substance, this interpretation is not conclusive. Even though thrombin in contact with alcohol precipitates slower than the other proteins in blood serum, it must still be admitted that thrombin solutions ex-

tions. To mention them would take us into a somewhat different field. A critical investigation of this question would tie in closely with the problem of the chemical nature of thrombin. It would have to deal also with entirely new factors which had been postulated by many investigators such as the clot-accelerating effect of tissue juice. One should also attempt to relate the role of the calcium salts in the activation of prothrombin to the theory of Alexander Schmidt regarding the origin of thrombin from "zymoplastic agents." Hammarsten called attention to this particular point, but it did not arouse interest for a long time. Schmidt's latest highly complicated theory of coagulation was largely ignored. The interest in it was stifled by the recognition of the theory concerning the role of calcium, and thorough, careful accounts like that by Arthus did not even mention Schmidt's coagulation theory.

erature—"cell-globulin," "cell or tissue fibrinogen," "nucleohistone," "nucleo-albumin precursor," "tissue nucleoprotein," "koagulins"—all refer to the same substance. The "zymoplastic agents" of Schmidt probably also belong to this group. The names listed above emphasize the divergent opinions of the respective authors concerning the nature of these substances, and show in retrospect why the study of the literature on coagulation is in some respects a rather unrewarding task. One hopes that newer studies which will be mentioned later will resolve these difficulties.

Schmidt's opinions concerning the function of these substances were expressed in his studies on the "zymoplastic agents" which were discussed previously. Schmidt did not consider these substances identical with thrombin. Rather he characterized the active principle as an alcohol-soluble, heat-stable substance which converted an inactive precursor into thrombin.

By contrast a number of investigators, particularly Halliburton and Pekelharing, considered the active principle of tissue juice to be thrombin or its precursor. Halliburton's and Friend's experiments (370) took up where Gamgee* left off. The latter had shown that fibrin clots yielded a globulin-containing fluid upon extraction with an 8 per cent sodium chloride solution. The fluid thus obtained possessed strong thrombic activity. Halliburton and Friend tried to prove the globulin character of thrombin by the isolation of a globulin from the tissues, the so-called "tissue globulin," which likewise possessed "fibrinoplastic" properties and which had the same characteristics as the globulin which Gamgee obtained from fibrin.

An active cell globulin preparation was obtained in large amounts by Halliburton (196, 197) from lymphocytes

* Gamgee, A.: Some old and new experiments on the fibrin ferment. *J. Physiol.* 2:145, 1879.

tracted from an alcoholic precipitate are less active the longer they have been in contact with alcohol, i.e., the more the proteins have been precipitated. The potency of thrombin solutions roughly parallels the protein content.

Investigations on thrombin solutions produced according to Schmidt's method, therefore led to no conclusion concerning the nature of thrombin. However, observations on the clot-promoting substances obtained from tissues seemed to favor the protein nature of thrombin.

It had been known for a long time that cells contain substances which initiated or accelerated coagulation. One of the earliest observations in this field stemmed from Naunyn's successful experiment in which he produced intravascular coagulation by the injection of laked blood (382, 383). Buchanan (19) had already observed the clot-accelerating effect of different tissues on plasma *in vitro*.

These observations concerning the clot-promoting substances in tissue cells were put on a sound experimental basis by the significant study of Rauschenbach (82) which was mentioned previously. This study had indicated that tissues containing many nuclei have particularly large amounts of such substances. Concurrently Foà and Pellacani (364) discovered that the injection of tissue juice from a large variety of organs produced intravascular coagulation. Only the splenic extracts were ineffective. For this reason both authors, like Rauschenbach, considered that thrombin was a general protoplasmic derivative.

Since then many authors have studied the clot-promoting substances in tissues. Depending on the different positions the various authors took concerning the theory of blood coagulation, the tissue substances were at times considered to be thrombin or its precursor, and at other times not. Just as numerous as the opinions were the names given to these substances. The many terms used in the lit-

cursor and to remove the globulins from it. The process was briefly as follows: Fibrinogen was precipitated from oxalate plasma by sodium chloride and the filtrate was dialyzed until it became turbid. Acetic acid was added until a weak acid reaction occurred and a precipitate formed. This was soluble in dilute alkali and neutral salt solutions, but, in contrast to globulin, not in dilute acids. This substance was considered to be the precursor of thrombin and when treated with calcium salts to yield active thrombin.

Using digestion with pepsin he apparently demonstrated that the precursor was "nucleo-albumin." In that process a precipitate formed which was insoluble in acids but readily soluble in alkalis and which yielded an ash rich in phosphorus. Therefore, the precipitate was thought to be nucleic acid. Pekelharing also mentioned two other similar methods for the isolation of the precursor. Like the original method these two attempted to separate the "nucleo-albumin" from the precipitated globulins by the addition of dilute acids in solutions of low salt content.

"Nucleo-albumin" was soluble in dilute solutions of sodium chloride but precipitated when cooled to 0° C, and thus could be separated from the rest of the globulins which remained in solution. This observation was consistent with the one previously made by Hammarsten. Therefore, according to Pekelharing thrombin was the calcium compound of "nucleo-albumin." No "nucleo-albumin" was found in solution in circulating plasma but was thought to originate from the formed elements, especially from the leucocytes and platelets. It was considered possible by using various anticoagulants (e.g., leech extract or sodium fluoride) to obtain a plasma which following the removal of formed elements by centrifugation would not yield a precursor or nucleoprotein when treated by the above

by extraction with sodium sulfate and subsequent precipitation with magnesium sulfate. Thus supposedly two globulins were produced: "globulin A" which coagulated at 50°C and had no thrombic activity, and "globulin B" which coagulated at 75°C and was identical with thrombin. Halliburton considered that an identical globulin could be extracted from the stroma of red cells which, as was well-known, promoted coagulation markedly (370). However, the proof of the thrombic nature of this globulin was not compelling. Halliburton pointed out analogies between cell globulin and thrombin in their reactions to heat, alcohol, salt precipitation, etc. Other cell globulins e.g., myosinogen of muscle tissue, were thought to possess thrombic activity. Therefore, different chemical substances were thought capable of acting as thrombin. Halliburton had the idea that during coagulation "cell-globulin-thrombin" was transferred from the cellular elements (especially from the leucocytes) into plasma and combined with fibrinogen.

Pekelharing (210), like Halliburton, identified the active substance of tissue juice with thrombin or at least with its precursor. But he did not ascribe to thrombin the chemical nature of a globulin. Rather he tried to prove that the precursor of thrombin was a "nucleo-albumin," (or as we say nowadays, a nucleoprotein) while active thrombin was a calcium compound of this protein. Pekelharing took an important step forward when he successfully isolated this "nucleo-albumin" not only from tissues but also from those specimens of blood plasma which could be assumed to contain prothrombin.

Pekelharing (210) fell back upon his earlier observation that oxalate plasma yielded a substance in the globulin precipitate which in turn produced thrombin in combination with calcium (170). He attempted to isolate this pre-

plastic" properties, but they believed that its effect was different from that of thrombin since it could not coagulate salt plasma but intravascularly had a much more pronounced effect than thrombin. It was apparent that Halliburton had changed his mind completely concerning the active substance of tissue juice which he now viewed as nucleoproteins and not as identical with thrombin. In a later publication Pekelharing (212) attempted to refute these objections and others raised by Wright (215).

Later Hammarsten (124) pointed out that thrombin may not be identical with the nucleoprotein-calcium complex of Pekelharing but may be merely mixed with it. This argument was justified since very potent thrombin solutions obtained from blood serum contained only traces of nucleoproteins, while Pekelharing's nucleoprotein obtained from tissue extracts possessed only minimal thrombin activity. Despite these objections Pekelharing and Huiskamp (213) insisted upon the thrombic nature of the nucleoproteins, and attempted to demonstrate that such substances obtained from the thymus solely by electrolytic methods showed considerable "fibrinoplastic" activity.

Apparent proof for the thrombic nature of the active component of tissue juice was obtained in the interesting investigations by Delezenne (409-413) concerning the coagulation of blood in birds, reptiles, frogs, and fish. It has been commonly assumed that the blood of birds coagulates very quickly. There are, however, statements in the older literature to the effect that blood coagulation in birds and reptiles occurs extremely slowly (see Alexander Schmidt [54] and Tiegel [447]). These statements remained unconfirmed. Delezenne was able to show that avian blood could be maintained fluid outside of the blood vessels without the addition of an anticoagulant, as long as the blood was removed through scrupulously clean cannulas

methods. This will be discussed at length later.

On the other hand oxalate plasma allegedly contained the precursor of thrombin. Therefore, oxalates and fluorides both of which precipitated calcium salts apparently worked by different mechanisms since only the fluorides simultaneously prevented the release of the precursor from the formed elements into the plasma.

Pekelharing also showed that "nucleo-albumins" obtained from tissue extracts, especially from extracts of thymus and testicle, displayed "fibrinoplastic properties" when combined with calcium. These "nucleo-albumins" were also considered by Pekelharing to be precursors of thrombin since in combination with calcium they could bring about coagulation both in extravascular and intravascular plasma. The "nucleo-albumins" of the tissues differed from each other and from the plasma "nucleo-albumin" in several respects, e.g., in their heat stability. They lost their "fibrinoplastic activity" at different temperatures. From this it was concluded that there was not just one thrombin but that the calcium salts of different "nucleo-albumins" could act as thrombin. Even the casein of milk could under certain conditions apparently show thrombic properties.

It can be seen that Pekelharing's theory was related to that of Lilienfeld in many respects. Both authors emphasized the importance of nuclear substances in coagulation. But Lilienfeld did not want his "leuconuclein" identified with thrombin or prothrombin (433).

Pekelharing's identification of thrombin with a calcium salt (to which Castellino [188] subscribed *in toto*) was not at variance with Hammarsten's idea of the role of the calcium salts in coagulation (165). But there were other objections to Pekelharing's theory. Halliburton and Brodie (371) admitted that "nucleo-albumin" possessed "fibrino-

blood obviously establishes contact quickly with the tissues which as noted previously brings about rapid coagulation. Do the tissues contain thrombin? Delezenne and subsequent investigators answered this question in the affirmative. Fuld used goose plasma and muscle extracts to study the time necessary for thrombin to produce coagulation, because the speed of coagulation, often less than one minute, promised more favorable experimental conditions than the use of artificial coagulation mixtures. Fuld (193) considered the possibility that muscle extracts contain "zymoplastic agents" rather than thrombin. However, he finally concluded otherwise since in his experience the active component of tissue juice was not heat-resistant, while Schmidt's "zymoplastic agent" could withstand boiling.

Using fluoride plasma as an indicator for the presence of thrombin, Arthus (348, 178, 179) arrived at different findings concerning the nature of the tissue components which promoted coagulation. Fluoride plasma (0.3 per cent sodium fluoride) was much better suited for this purpose than oxalate plasma since fluorides in low concentration impaired the effect of thrombin very little. Arthus then confirmed the finding that blood clotted much faster on extensive contact with tissue or when it flowed over the skin, than when it was collected into a glass tube directly from the blood vessel. Likewise tissue extracts greatly accelerated the coagulation of blood *in vitro*. What did the tissues contribute to the blood? According to Arthus tissue extracts certainly did not contain thrombin since they were unable to coagulate fluoride plasma which clotted readily upon the addition of thrombin. Nor did tissue extracts apparently contain prothrombin, because they remained inactive even after the addition of calcium salts. Up to this point Arthus' reasoning was convincing. Then,

and collected in completely dust-free, carefully cleaned containers. Centrifugation of the blood yielded a plasma which remained fluid for a very long time, often until the onset of decomposition. While whole blood would readily clot in the presence of small particles of dust and other impurities, the cell-free plasma was much more resistant to these influences. By contrast blood as well as cell-free plasma clotted with extraordinary rapidity when mixed with a very small amount of tissue juice. However, as noted previously, blood would coagulate by itself without the addition of tissue juice. In centrifuged or sedimented blood, coagulation began in the leucocyte layer. Blood from reptiles (41), frogs, and fish (412), i.e., the blood of all animals with nucleated red cells, reacted similarly. When, however, the blood of vertebrates was treated in the same manner as avian blood, coagulation was only negligibly retarded (413). Delezenne assumed that leucocytes and the other formed elements of oviparous animals were more resistant to damage by external factors than those of mammals, and that they released thrombin into plasma only after a strong stimulus. This assumption appeared to be quite correct.

The important aspects of Delezenne's results were soon confirmed by Phisalix (440), Spangaro (287), and Fuld (193). Avian plasma provided a new tool for the solution of numerous experimental problems. I was able to demonstrate that it can be easily obtained. This is more than can be said for the analogue of avian plasma, i.e., mammalian plasma kept fluid in paraffined tubes according to the method of Freund. Geese are to be recommended. Blood can be readily obtained from the carotid artery or still better from the jugular vein. Chickens often perish during the blood-letting.

During the clotting of avian blood following injury, the

significance of the formed elements of blood (454). Plasma, according to Wooldridge, contained all the factors necessary for coagulation. These factors he called "fibrinogen-A" and "fibrinogen-B" which combined under certain circumstances to form fibrin. "Fibrinogen-A" was obtained as a granular precipitate from peptone plasma after prolonged cooling at 0°C. It probably corresponded at least in part to what other authors have called platelets. Wooldridge considered that his "fibrinogen-B" in its natural state in plasma was a precursor of Hammarsten's fibrinogen but not identical with the latter. This was based on the assumption that thrombin would not clot unaltered "fibrinogen-B" in plasma but would clot Hammarsten's fibrinogen because the latter had been modified by repeated salt precipitation. Wooldridge did not question the existence of thrombin but considered it a result rather than the cause of coagulation.

Wooldridge thought that coagulation was actually brought about by the fibrinogens, especially "fibrinogen-A," and its active analogues, "tissue fibrinogens." The latter were obtained by extraction of tissue with dilute sodium chloride solution and subsequent precipitation with acetic acid. They were considered simply as elements of tissue, and Wooldridge theorized that the bulk of protoplasm consisted of such "fibrinogens." According to Wooldridge they deserved the name "fibrinogens" just as much as did "fibrinogen-B" of plasma since he thought they entered into the complex of formed fibrin and were therefore used up during coagulation.

Both the fluid state of blood within the vessels and blood coagulation were considered to be the results of the interaction between the "fibrinogens," in which two phases were to be distinguished, a negative and a positive one. The negative phase was thought to predominate in cir-

however, he attempted to explain the effects of the thermolabile clot-promoting substances of tissue by assuming that tissue extracts possessed the power to initiate and accelerate the release of thrombin from the white blood cells. This was, however, an inadequate characterization of the importance of tissue juice, because, as Hewlett (201) had correctly observed, tissue juice also retained its effectiveness in completely cell-free plasma such as goose plasma or that prepared from peptone plasma. Thus, only the negative part of Arthus' publications was convincing.

Later Loeb (375) defended the thrombic nature of the active tissue substances, which he called "koagulins." They were not considered completely identical with thrombin as obtained from blood serum. They were thought to possess a greater specificity, but their overall effect was considered to be the same as that of thrombin. Therefore, Loeb assumed the existence of several active substances all of which could have thrombic activity.

The preceding paragraphs have dealt with the observations of those investigators who assumed the active component of tissue juice to be thrombin. Other studies which represented a different point of view and in some respects deviated considerably from the prevailing concepts should also be mentioned. Schmidt's concept of the "zymoplastic agents" and Lilienfeld's theory have already been discussed. There were also the studies of Wooldridge and Wright which will be discussed briefly. It is hardly necessary to go into the details of Wooldridge's peculiar theory of coagulation since Wooldridge never had a serious disciple except for Wright, and his theory has long since been abandoned. Wooldridge's theory, at least in its final version, differed from all theories promulgated since the time of Alexander Schmidt in its denial of the participation of thrombin in coagulation and its refusal to acknowledge the

of thrombin whose origin from cellular elements had been demonstrated by Pekelharing's observations. Furthermore, the same workers emphasized that one cannot readily generalize from experiments with peptone plasma because of the highly complex conditions found in this plasma. This was undoubtedly the reason why Wooldridge consistently interpreted his own accurate observations incorrectly, as will be pointed out in our discussion on peptone plasma.

Wright (215, 393-396) subsequently attempted to extend Wooldridge's theory by more precise investigation of the changes which occurred in blood after the injection of "tissue fibrinogen" (or "cell fibrinogen" as he called it). This will be discussed later. According to Wright, thrombin was a mixture of "cell fibrinogen" and calcium salts (395). Incidentally, at a later date, Wright considered that the clot-promoting substances did not actually originate from the cells but came from the contaminating lymph. Since it was stated in very general terms, this hypothesis was certainly not acceptable.

The preceding account concerning the clot-promoting substances in tissue juice illustrates the far-reaching and apparently irreconcilable discrepancies in the opinions of different authors. Pekelharing (210), Delezenne (409-413), and others claimed that tissues contained thrombin or its precursor. Schmidt (54), Wooldridge (454), Lilienfeld (433), and Arthus (348) felt that the tissues did not contain thrombin but rather substances which promoted coagulation by others means. Again there was considerable difference of opinion regarding the details of the process.

Pekelharing (210), Halliburton (371), and Lilienfeld (433) considered the active substances of tissues to be nucleoproteins. Schmidt thought they were alcohol-soluble substances, and Wooldridge considered them fibrinogen-like compounds. Pekelharing (210), Delezenne (409), and

culating blood. This reason was given for the observation that thromboses did not always develop after the injection of "tissue fibrinogen" into the blood stream. More often the blood temporarily became incoagulable which was considered an illustration of the negative phase in the interaction of the fibrinogens. However, larger amounts of "tissue fibrinogens" caused intravascular coagulation, primarily in the region supplied by the portal vein. *In vitro* in peptone plasma only the positive phase of fibrinogen interaction was observed. Peptone plasma clotted very quickly on the addition of "tissue fibrinogen," even after the removal of "fibrinogen-A." Both substances, as well as "serum fibrinogen" which was obtained from blood serum by acid precipitation, had the same effect. In normal coagulation it was thought that "fibrinogen-A" combined with "fibrinogen-B." "Tissue fibrinogens" did not bring about clotting in some exudates even though the latter contained "fibrinogen-B." This finding was simply ascribed to an alteration in the fibrinogen of these transudates. "Fibrinogen-B" was thought to occur in many different forms and by no means to be as constant in its properties as Hammarsten had assumed.

The discussion of some of the studies (448-454, 391, 392) which helped Wooldridge in the development of his theory would carry us too far afield. His earlier studies emphasized the significance of lecithin in coagulation in that "fibrinogen-A" was thought to transfer lecithin to "fibrinogen-B." Wooldridge apparently abandoned this idea later.

Wooldridge's theory of coagulation did not attract followers and was soon completely abandoned after his premature death. Its lack of appeal was due chiefly to the objections of Halliburton (197), Pekelharing (210), Krueger (77), and others, who pointed out that the "tissue fibrinogens" and "fibrinogen-A" contain nothing but a precursor

active form. The serum form is not activated by calcium salts but rather by alkalis and acids, even in the complete absence of calcium salts. Fuld (194) and I demonstrated this in support of Schmidt's assertions. This "pro-enzyme" (which I originally called "β-prothrombin," while Fuld named it "metazyme") is found only in serum and is absent in all unclotted plasma samples, e.g., oxalate and fluoride plasma. Thus it appears reasonable that the inactive form of thrombin is not so much a *pro*thrombin, but a modification into which thrombin is converted quickly following coagulation. This idea is supported by the observation that serum contains surprisingly little free thrombin. Shed blood coagulates in a few minutes although thrombin has to be formed first. The thrombic activity of relatively fresh serum is, however, so weak that it requires at least a half hour to clot a fibrinogen solution. During coagulation a large amount of thrombin adheres very firmly to the fibrin and is removed with the latter. Nevertheless, shortly after coagulation a considerable, or indeed the largest, portion of the formed thrombin is probably converted into an inactive form which can be re-activated by alkalis or acids. The term "metathrombin" seems appropriate for this substance. The manner in which metathrombin is formed from thrombin is not known. Condensation, polymerization, or similar processes are possible mechanisms.

The chemical properties of metathrombin, e.g., its reaction to heat, are about the same as those of thrombin, but it is much more resistant to the damaging effects of light, air, etc. When serum in an uncovered container is exposed to air for several days until its thrombin has completely vanished, it still contains large amounts of metathrombin.

Thrombin which is obtained from metathrombin by activation with alkali quickly deteriorates again and loses

Conradi (358) felt that the active substances were thermolabile, while Schmidt and Wooldridge were unable to destroy their activity by boiling.

These vast differences in opinion were also expressed in the two most important theories concerning the origin of thrombin, i.e., in the theory of Alexander Schmidt and in that of Pekelharing and Hammarsten. The latter theory (formerly the predominate one) held that the calcium salts of plasma activated a precursor of thrombin. This precursor, a nucleoprotein, was considered to be released from the formed elements into the plasma after blood was shed. By contrast, Schmidt assumed that prothrombin already existed in the circulating plasma, and that it was activated by "zymoplastic agents" without the participation of calcium salts. The "zymoplastic agents" were thought to be released from the leucocytes only after blood was shed. Free alkali allegedly augmented this process considerably.

Naturally it was highly desirable to resolve these contradictions as much as possible. Fuld (194), Morawitz (207, 208), and Fuld and Spiro (195) undertook this task. *They arrived independently at results which were identical in all essentials and which helped to eliminate a considerable number of the misunderstandings.*

My first paper showed that in part the difference of opinion concerning the effect of calcium existing between Schmidt on the one hand, and Arthus, Hammarsten and Pekelharing on the other, undoubtedly resulted from the fact that the prothrombin of Schmidt was not identical with that of the other authors. There seem to be at least two inactive forms of thrombin. One is found in oxalate plasma and is activated by calcium salts alone. This was Pekelharing's prothrombin, for the time being referred to as "α-prothrombin." Another inactive form of thrombin is found in blood serum in large quantities along with the

extraction of the tissue. If allowed to stand for any length of time, such extracts lose their activity even when decomposition is avoided by the addition of preservatives. However, after drying *in vacuo* powdered tissue extracts retain their activity for a long time.

Do tissue extracts contain thrombin or Pekelharing's precursor? The experiments of Fuld and Spiro (195) as well as my own observations (208) demonstrated that neither was the case. Tissue extracts from avian liver were not capable of coagulating Hammarsten's fibrinogen solution even in the presence of calcium salts. Serum produced by the spontaneous clotting of goose blood, on the other hand, possessed that ability. It was also not possible to clot fibrinogen solutions, oxalate plasma or fluoride plasma by the addition of mammalian tissue extracts whether calcium salts were present or not. Likewise some transudates such as hydrocoele fluid could not be clotted by similar treatment.

However, the coagulation of whole blood was greatly augmented by the addition of tissue extracts. These extracts also initiated coagulation in goose plasma, peptone plasma, and occasionally in leech extract plasma, but only in the presence of calcium salts. In decalcified goose and peptone plasma the tissue extracts were completely inactive.

The thrombic activity of blood serum was greatly enhanced by the addition of tissue juice. An increase in the alkalinity of serum had a similar effect. Despite these suggestive findings it did not appear likely that it was actually metathrombin which was activated by the added tissue juice. After all, tissue juice was active only in the presence of calcium, whereas metathrombin could be activated to thrombin by alkali in decalcified serum.

On the basis of these and other less important observations omitted from this discussion, Fuld and Spiro and I

its activity, especially in alkaline media and at higher temperatures. Whether this constitutes an important difference between re-activated methathrombin and the thrombin formed during coagulation remains questionable. Nor do we know whether thrombin which has been obtained from metathrombin can be re-converted to the metathrombin stage. According to personal observations this does not appear likely. Another striking and unexplained fact is the retarding effect of normal serum on the alkali-activated metathrombin. It is possible that this is an inhibitor effect.

While proof of the existence of two different inactive forms of thrombin reconciled some of the apparent contradictions between the statements of Schmidt and those of later investigators, clarification of the role of the "zymoplastic agents" was still needed. It was necessary to determine whether "zymoplastic agents" really existed, and if so which of the inactive precursors of thrombin they acted upon. The relationship of calcium salts to the effect of the "zymoplastic agents" also requires clarification.

There are very few references to Schmidt's "zymoplastic agents" in the subsequent literature. Lilienfeld (433) thought that their effect could be attributed to the phosphoric acid content. Spiro and Ellinger (289) claimed to have brought about the coagulation of peptone plasma by the addition of "zymoplastic agents." My own efforts to duplicate Schmidt's isolation of "zymoplastic agents" from dried alcoholic cell extracts have been unsuccessful. So far no explanation has been found for the results which Schmidt obtained.

On the other hand, it was easy to prove the existence of clot-promoting, heat-labile substances in all tissues, especially in those rich in cell nuclei such as the thymus and lymph glands as well as in the stroma of the red cells. These substances can be readily obtained by simple saline

tion in circulating plasma but was produced *in vitro* from the formed elements, chiefly the platelets (480).

These arguments were nullified by the subsequent studies of Bordet and Gengou (186). These investigators showed, like Fuld (194), that fluoride plasma contained thrombogen which was relatively completely adsorbed and bound by the massive precipitate of calcium fluoride which incidentally also removed a portion of the fibrinogen. These findings compelled me to modify my previous opinions. Furthermore, the observations of Arthus (182) which denied the pre-existence of thrombogen in circulating blood were no longer convincing. Arthus pointed out that many transudates did not contain thrombogen, a fact which Alexander Schmidt and Rauschenbach (82) had long recognized. It could be assumed that the activity of thrombogen was rather quickly lost in these isolated fluids unless a further supply was constantly transferred into the transudates from the blood.

Proof that thrombogen is present in fluoride plasma also influenced my investigations concerning the origin of this substance (480). As noted previously thrombogen has not been consistently demonstrated in the tissues investigated. On the other hand I observed that thrombokinase as well as thrombogen could be found in platelets obtained by differential centrifugation of fluoride plasma. Since thrombogen had been found in fluoride plasma these experiments were no longer convincing, especially since other personal observations yielded evidence against the platelets being the source of thrombogen. Furthermore, in experimental phosphorus poisoning thrombogen occasionally disappeared completely from the blood (93), but platelets remained in appreciable concentrations and showed no microscopic abnormalities.

It must be regretfully admitted that thus far no defini-

concluded that tissue juice contains a substance which leads to the production of thrombin in the presence of calcium salts and of another substance which is also present in goose plasma, peptone plasma, and serum. Fuld called the active "zymoplastic agent" of the cells "cytozyme," while I have referred to it as "thrombokinase." The substance in plasma has been named "plasmozyme" or "thrombogen,"* respectively. In the following paragraphs my terminology will be tentatively employed without necessarily intending to anticipate a more definitive nomenclature.

Thrombokinase can be obtained from all forms of protoplasm and from blood cells, especially platelets and leucocytes. Thrombogen, on the other hand, has so far been found only in plasma. Since thrombogen is present in all artificial plasma specimens, Fuld assumed its prior existence in a soluble state in circulating plasma. Originally I felt that I had found a thrombogen-free but active solution in fluoride plasma since the investigations of Pekelharing (170) and of Arthus (178) had shown that the effect of fluoride was different from that of oxalate. It was thought that oxalate plasma clotted readily upon the simple addition of $CaCl_2$ because it contained a precursor of thrombin. By contrast fluoride *blood* clotted after the addition of $CaCl_2$ only if diluted with distilled water. Fluoride *plasma* remained liquid under the same circumstances. From this Pekelharing and Arthus concluded that fluoride, in addition to precipitating calcium, fixed the leucocytes so that they were unable to release prothrombin into the plasma. I demonstrated that fluoride plasma occasionally remained liquid after the addition of calcium salts or tissue juice even though active thrombin readily clotted it. The tentative conclusion was that thrombogen was not found in solu-

* *Translators' note:* In modern terminology "prothrombin" is used.

produce thrombin. As soon as thrombin has been produced, calcium is no longer needed for coagulation. Therefore, thrombin results from the interaction of at least three substances.

In the process of thrombin formation the supply of thrombogen is never completely exhausted. A considerable quantity of it is found in serum after coagulation has taken place and can be re-activated by the addition of tissue juice. After coagulation, a sizable portion of the thrombin produced is quickly converted into the inactive form, metathrombin, which can be converted into thrombin again by means of alkali or acid. The entire process of thrombin formation according to Fuld (194) is graphically presented in Figure 2. This outline contains some points which have not been proved, but it presents the known facts in the best possible manner.

The presence of thrombin precursors in various body

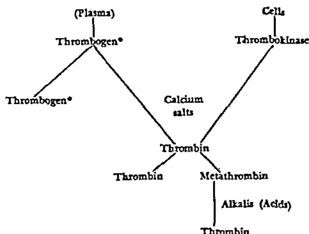


FIG. 2. * *Translators' note:* The popular modern term is "prothrombin."

tive information has been obtained concerning the source of thrombogen or the pre-existence of the latter in circulating blood. According to Fuld, the pre-existence of *thrombogen in circulating blood is highly probable*, particularly since thromboses occur following the injection of thrombokinase. Perhaps detailed hematological investigation of the thrombogen-free blood produced by phosphorus poisoning will clarify this matter.

The question regarding the origin of thrombokinase can be answered more readily. Its chief source in mammals is probably from platelets whenever coagulation occurs normally. It is not simply a coincidence that fluids which do not contain platelets (e.g., lymph and possibly oviparous blood) coagulate only very slowly even under favorable conditions or else remain entirely liquid. It seems reasonably safe to assume that thrombokinase in such fluids originates from the leucocytes which release the active substances much more slowly than the very labile platelets do.

Thus the process of coagulation, according to the ideas of Fuld and Spiro (195) and myself, can be formulated approximately as follows: *Fibrinogen, calcium salts, and probably also thrombogen exist in the plasma of circulating blood. Outside of the vessels the formed elements, especially the platelets, are disrupted by contact with foreign surfaces and therefore yield thrombokinase into the plasma. Thrombokinase in conjunction with thrombogen and calcium salts forms thrombin.* Because the chemical nature of enzymes is unknown, we have no detailed knowledge concerning the interaction of these three substances. We do not know whether thrombokinase is incorporated into the thrombin molecule in the manner of an ambocepter nor the role played by the calcium salts. At any rate, it is certain that calcium salts must be present at the moment that *thrombokinase and thrombogen interact to*

parently erred in identifying metathrombin with prothrombin. That metathrombin could be activated in the absence of calcium salts probably explains Schmidt's denial of the importance of the calcium salts. The results Schmidt obtained with alcoholic cell extracts remain unexplained.

Those investigators who believed that tissue extracts contained thrombin or its precursor which could be activated by calcium salts, used substrates which contained prothrombin such as goose plasma and peptone plasma in their experiments. This explanation, however, does not apply to the results obtained by Pekelharing and Huiskamp (213). These investigators were able to coagulate fibrinogen solutions prepared by Hammarsten's method by adding nucleoproteins from the thymus gland. It can be assumed that their fibrinogen solutions were not completely free of prothrombin. Moreover, thymic extracts frequently contain small amounts of prothrombin which might have originated from the blood or lymph remaining in the tissue. Frequently, however, they contained no prothrombin in which case even very potent thymic extracts did not initiate coagulation of a fibrinogen solution despite the presence of calcium salts.

Schmidt's prothrombin is without doubt identical with thrombogen. Pekelharing's concept of prothrombin included thrombogen and thrombokinase without calcium salts. Since Pekelharing's prothrombin consisted of two factors, its nucleoprotein nature is naturally doubtful. It is nevertheless quite possible that thrombokinase is a nucleoprotein. It shows striking similarities to this type of protein and by prolonged cooling can apparently be completely precipitated with the nucleoproteins from peptone and oxalate plasma. This precipitate ("fibrinogen-A," prothrombin) was observed by Wooldridge (454), Hammarsten (165), and Pekelharing in a finely granular form re-

fluids is shown in Table I. It becomes clear from the preceding considerations that circulating blood remains liquid because of the absence of thrombokinase, or because the latter is liberated so slowly that it is rendered inactive by anticoagulants. This phenomenon will be discussed in a subsequent chapter.

The preceding account shows that by and large there

TABLE I

	*Thrombogen**	*Thrombokinase*	*Thrombin*	*Metathrombin*
Circulating plasma	+?	–	–	–
Exudates (ascites)	+	–	–	–
Hydrocoele fluid	–	–	–	–
Oxalate plasma	+	+	–	–
Goose plasma	+	–	–	–
Serum	+	+?	+	+
Thrombin solution of Schmidt	?	?	+	–
Fluoride plasma	+	–	–	–

* *Translators' note:* The popular modern term is "prothrombin."

exists a striking analogy between modern ideas concerning the origin of thrombin and Alexander Schmidt's little-recognized theory of the "zymoplastic agents." Thrombogen is identical with Schmidt's prothrombin* and thrombokinase with the "zymoplastic agents." But Schmidt ap-

* *Translators' note:* Although Morawitz subsequently used the term "thrombogen" in this monograph, we have chosen the more popular modern term "prothrombin."

contact with non-paraffined surfaces, i.e., with wettable surfaces. It is clear that the effect of contact with the glass surface was not mediated through the disintegration of the formed elements, because there were none left in the plasma. Nor did contact with the glass surface set off the effect of any "pre-formed" thrombin upon fibrinogen since there was no active thrombin in "paraffin plasma" a fact which could be demonstrated by the removal of calcium salts. Decalcified "paraffin plasma" no longer coagulated upon contact with foreign bodies. These experiments showed that the production of thrombin from its plasmatic precursors is initiated or at least enhanced by contact with foreign surfaces.

In my opinion the experiments of Bordet and Gengou (185) are extremely significant in explaining the liquid state of blood. It is most likely that some thrombokinase is constantly escaping into the circulating plasma. This would produce fatal thromboses if the body did not possess a mechanism to counteract such danger. One of the safeguards may be the absence of contact with wettable surfaces in the circulation. Other safeguards and defense mechanisms will be discussed later.

The preceding outline of the production of thrombin according to the studies of Fuld and Spiro (195) and myself (208) might be criticized because the theory appears extremely complicated. Of course, it was not intended to be final and definitive. It must be admitted that for the time being this version best explains the observed phenomena. Above all it has the great advantage of reconciling the contradictory views of different scholars.

That this theory has been challenged cannot be denied. Loeb stoutly upheld the importance of the tissue "koagulins" as thrombins and doubted the significance of the calcium salts (436). As far as the first point is concerned,

sembling platelets. This provides another clue concerning the origin of thrombokinase. There is considerable evidence against the identification of thrombokinase with nucleoproteins. However, the striking fact remains that, according to Pekelharing and Fuld, body fluids which do not contain thrombokinase also lack cold-precipitable nucleoproteins. The same applies to goose plasma, fluoride plasma, and leech extract plasma. While this may not be just coincidence, there need not necessarily be a causal relationship. The disruption of formed elements may explain this phenomenon.

Prothrombin certainly has nothing to do with nucleoproteins and cannot be precipitated by cooling. Just as puzzling as the nature of the precursor of thrombin is the mode of action of thrombokinase. Thus far no one has been able to determine whether the reaction is enzymatic or stoichiometric. My personal observations support the latter possibility (328). It must be admitted that the matter is not settled.

The presence in plasma of prothrombin, thrombokinase, and calcium salts does not suffice for the formation of thrombin. A fourth factor is needed, namely contact with a wettable surface. This is important not only for the release of thrombokinase from the formed elements but also for the origin of thrombin in the plasma. This was demonstrated by the interesting experiments of Bordet and Gengou (185) which I have been able to confirm. When blood was collected in paraffined containers according to Freund's method (161), prolonged centrifugation yielded a plasma which was completely devoid of formed elements and platelets although the latter probably partially disintegrated during the preparation of the plasma. This plasma remained liquid as long as it was in contact with paraffined surfaces only. However, it quickly coagulated in

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Loeb's arguments were not well founded, at least not in the case of the vertebrates. It is preferable not to enter into a discussion of the matter at this point. The role of the calcium salts, according to the observations of Fuld (194) and myself (208), is diametrically opposite to that ascribed to them by Loeb. Further experimentation is necessary to clarify these discrepancies.

At this point a few remarks can be inserted concerning the specificity of thrombin and of its precursors. Duclaux (3) thought that thrombin did not possess a species specificity in the vertebrates. Bordet and Gengou (302) and Fuld (193) took issue with this view. The experiments of Loeb (375) showed that Duclaux's theory was correct and that specificity existed only between vertebrates and invertebrates. By contrast thrombokinase possesses a distinct specificity within the vertebrates. Loeb's observations were completely confirmed by Muraschew (209). Other scattered references to thrombin lacked coherence and, unless they dealt with morphological problems or anticoagulants, could not be organized under specific headings.

A very interesting phenomenon which has not been sufficiently clarified is the shortening of the clotting time following massive hemorrhage—a matter of great physiological significance. This phenomenon had already been recognized by Alexander Schmidt and was more recently investigated by Arloing (398), Arthus (401), and Milian (438). It can be readily ascertained that this change in the clotting time was considerable and certainly not due to faulty technique. The clotting time often shortened from seven minutes to less than one minute. It might be assumed that this remarkable phenomenon is based on changes in the production of thrombin, but the details are unknown. This protective mechanism is said to be absent in some cases of hemophilia, even when the patient had a normal baseline clotting time. This has been held

responsible for the great difficulty encountered in controlling hemophilic bleeding.

Another interesting phenomenon is the coagulation behavior of cadaver blood. The cause has not been sufficiently studied. Pathologists know that the blood in the vessels of a cadaver at times clots extensively, and at other times remains essentially fluid. When removed from the vessels, the blood in most instances coagulates although very slowly. Earlier investigators, who were still under the influence of the theory of the "fibrinocrasias,"* placed great emphasis on the amount and arrangement of blood clots in the cadaver (Wunderlich [40]).

As far as I know, Schmidt is the only one who devoted a brief discussion to this phenomenon and its etiology. He believed that the leucocytes in the vessels of the cadaver die without releasing their thrombin or "zymoplastic agents" into the plasma. Current knowledge suggests additional possibilities. The problem is certainly worthy of experimental study.

An important question concerns the influence of the amount of thrombin upon the clotting time. Problems in methodology make this difficult to solve. Fuld (193) alone presented precise data concerning this particular field. Previous investigators such as Duclaux (3) and Arthus (178) assumed a direct proportionality. Fuld, however, showed that doubling the amount of thrombin while keeping the amount of fibrinogen constant only accelerated the coagulation time one and one-half times. This applied only to large amounts of thrombin. With a small concentration of thrombin and a long coagulation time, the influence of the amount of thrombin was much more pronounced, and an almost direct proportionality existed.

Fuld's observations without doubt furnished further

* *Translators' note:* This term can be best defined as implying pathological alterations in the blood clot.

proof for the enzymatic nature of the coagulation process. However, they cannot be unequivocally utilized as a demonstration of the thrombin clotting time, because Fuld used muscle juice for his thrombin solution and goose plasma for the substrate. He did not then know the nature of muscle juice and thought it contained thrombin. The clotting times he observed concerned thus not only the time required for the second stage but also for the first stage of coagulation (i.e., the production of thrombin). A repetition of the experiments using thrombin and fibrinogen solutions would be desirable.

Arthus (180) provided data concerning the time necessary for the formation of thrombin in shed blood. He stopped the formation of thrombin in shed blood at various intervals of time by the addition of sodium fluoride and compared the amounts of thrombin produced. He observed a rapid increase in thrombin just before the first appearance of coagulation which lasted until shortly after the appearance of the first clots.

Chapter V

FIBRINOGEN AND FIBRIN (THE SECOND PHASE OF BLOOD COAGULATION)

HAMMARSTEN's investigations which had been repeatedly confirmed, clearly demonstrated that the second phase of coagulation consisted of the transformation of a plasma protein, fibrinogen, into an insoluble form, fibrin, by the action of thrombin. No mode of formation of fibrin other than by the action of thrombin is known.

According to Hammarsten fibrinogen should be classified *with the globulins (120, 121) since it is insoluble in* water, soluble in dilute solutions of neutral salts, and precipitated from plasma during dialysis. It is distinguishable from the other globulins of blood plasma by its lower temperature of "heat-coagulation." It is precipitated from weak salt solutions and from blood plasma at 56°C, and from an 8 to 10 per cent sodium chloride solution at 52° to 55°C according to Hammarsten and Frédéricq (112).

Furthermore, fibrinogen is more easily salted out by sodium chloride than are the globulins. The bulk of fibrinogen is precipitated by 50 per cent saturation with sodium chloride. This was the basis for the method of isolating fibrinogen in a pure state which was first used by Hammarsten. In contrast to the globulins fibrinogen completely precipitates upon 100 per cent saturation with sodium chloride.

According to Hammarsten the empirical formula of fibrinogen is: C, 52.93 per cent; H, 6.9 per cent; N, 16.6

per cent; S, 1.25 per cent; O, 22.26 per cent. Its C, H, and O content is somewhat higher than that of its product, fibrin. According to Mittelbach the optical rotation of fibrinogen is α (D) = minus 52.5° (142).

Fibrinogen has a tendency to become insoluble when precipitated from solution, particularly when precipitated with water or dilute acids. Furthermore, in preparing fibrinogen by the salt precipitation method a considerable amount may be lost due to its insolubility. The preparation of a fibrinogen solution which has to be purified three or four times is a difficult undertaking as my own experience has shown. For the technique of producing a fibrinogen solution see the studies of Hammarsten (165) and Morawitz (207). Insoluble fibrinogen is by no means identical with fibrin.

Whether fibrin can be considered a protein in the strict sense of the term is doubtful. As Schwalbe (11) pointed out, in contrast to other proteins it did not pass through a Chamberland filter but remained behind in the form of a sticky sediment which soon plugged the filter pores completely. The most striking property of fibrinogen is its transformation into fibrin by the action of thrombin. Hammarsten established the fact that fibrinogen is the sole precursor of fibrin.

This emphasizes the physiological importance of fibrinogen and the reason that the origin and production of this protein have been studied with great interest for a long time. Two opposing theories have dominated this field. Prévost and Dumas (35), and more recently Heynsius (128, 129) and Mosso (143), championed the earlier belief that fibrinogen or soluble fibrin, was not present in circulating plasma but originated in shed blood by the release of a protein from the red blood cells. Mosso on the basis of microscopic observations claimed that the red cells

lost their pigment and subsequently fused into a hyaline mass, the fibrin coagulum. Heynsius thought he found 91 per cent of the fibrin in the red cell layer of sedimented horse blood and only 9 per cent in the plasma. These observations resulted from the faulty methods of identifying fibrinogen and fibrin in use at that time. Alexander Schmidt proved conclusively that the red cell layer actually contained very little fibrin.

Undoubtedly a fibrinogen-like substance can be extracted from red cells with dilute salt solution especially from the nucleated red cells of the lower vertebrates. Landois (134), called this "stromafibrin" and Semmer, a disciple of Schmidt, investigated this substance (87). This "stromafibrin" was also found after freezing and thawing of the blood cells, and after hemolysis in heterologous serum.

A similar hyaline, fibrous substance was isolated from certain leucocytes. There was, however, no proof that these substances were identical with fibrinogen. Landois thought that "stromafibrin" and plasma fibrin were different substances. This was undoubtedly true since this thick, tenacious and fibrous material consisted mainly of nucleoproteins. The same can be said of those substances which Wooldridge (454) collectively called "tissue fibrinogens," whose significance has already been discussed. The nature of the fibrinogen which Semmer (87) isolated from the blood cells of frogs is a bit obscure to say the least.

In addition to the red cells, leucocytes and platelets have also been considered as possible sources of fibrinogen. Morphologists, in particular, have alluded to observations which seemed to indicate that fibrinogen or fibrin originated from extravascular disintegration of these cellular elements. Buerker (460) very recently restated the same opinion. He attempted to relate fibrin to the products of

extravascular disintegration of platelets. He tried to demonstrate that the amount of fibrin formed depended upon the number of available platelets, and that it was mathematically possible that all of the fibrin could be derived from the sum total of the platelets.

The idea that fibrin originates from extravascular disintegration of the formed elements, denying as it were the pre-existence of fibrin in circulating blood, is essentially incorrect. Nowadays this concept is probably not upheld by a single chemist. It is acceptable only insofar as numerous histological observations have shown that in normal blood coagulation some of the cellular elements, especially the platelets, disintegrate and are incorporated as a hyaline or granular material into the mass of fibrin which is thus increased in bulk. However, there has been no proof that those granular masses are really fibrin and this seems highly improbable. For the time being we must consider these granular masses as impurities.

Furthermore, all other observations support the contention that fibrinogen already exists as such in the circulating plasma. From the time of the well-known experiment of Johannes Mueller (33) this theory has been commonly accepted. Actually, it is not at all difficult to prove that the circulating plasma must contain fibrinogen. A large accumulation of quantitative data has shown that the amount of fibrinogen in plasma probably does not increase appreciably in shed blood.

The pre-existence of fibrinogen in the plasma can be proved in many ways. The fact that various hypocellular transudates (especially equine peritoneal and pericardial fluid) contain fibrinogen supports this conclusion. Fluoride plasma contains a normal amount of fibrinogen even after it has been completely freed of all formed elements by centrifugation. Since leucocytes and platelets are well-pre-

served in fluoride plasma, there is no extensive breakdown of the cellular elements to release fibrinogen. There is no point in providing additional support for a fact which must now be considered completely established.

But if fibrinogen does not result from extravascular disintegration of the formed elements, the question remains: Where does it come from? This is intimately associated with the problem of the origin of the blood proteins in general which has not yet been solved.

Alexander Schmidt's ideas concerning the origin of fibrinogen have already been mentioned. Schmidt felt that fibrinogen originated from intracellular "cytoglobin" through the intermediate step of "paraglobulin." This remains mere hypothesis. There has been no further supporting data for the origin of fibrinogen from "paraglobulin" except that Dastre observed that fibrinogen can assume globulin-like properties under certain circumstances (96). Most observations do not favor the proposition that fibrinogen as such originates from the cellular elements.

More recent studies appear likely to yield specific information concerning the origin of fibrinogen. The first deals with those conditions in which fibrinogen is totally absent in circulating blood in which case blood naturally does not coagulate. Incoagulable blood prepared by certain methods (e.g., peptone blood, leech extract blood) contains a normal concentration of fibrinogen. The incoagulability of such plasma specimens is therefore not due to a lack of the substrate of coagulation. Only under very specific experimental conditions is fibrinogen lacking in the blood.

This occurs first of all in experimental phosphorus poisoning, as shown by the investigations of Corin and Ansiaux (93), Jakoby (132), and Loeb (436). Experiments in dogs showed that as phosphorus intoxication progressed, fibrinogen gradually disappeared from the blood.

Finally, shortly before death no fibrinogen was left, especially if the poisoning was not acute but was prolonged over a period of eight to fourteen days. The blood became completely incoagulable. Indeed, old, partially healed wounds frequently started to bleed vigorously again. This suggested that the blood in phosphorus poisoning was capable of dissolving thromboses. Indeed, Jakoby found that its fibrinolytic activity was greatly increased. Two reasons were given for the incoagulability of the blood in phosphorus poisoning. Not only was fibrinogen considered absent but there was also an approximately equal reduction in the concentration of prothrombin (the precursor of thrombin) whereas thrombokinase remained normal. Therefore, blood may still contain some fibrinogen and yet not clot spontaneously, but such blood coagulates on the addition of thrombin. The other blood proteins, especially the globulins, are not materially reduced in phosphorus poisoning as I have been able to demonstrate repeatedly.

These observations apparently indicated that the liver plays an important role in the formation of fibrinogen since it suffers the greatest functional damage in phosphorus poisoning. The same occurred in chloroform poisoning in which fibrinogen is likewise supposed to be absent. For the latter finding we are indebted to Doyon who considered the liver the primary source of fibrinogen, especially since he could extract a fibrinogen-like substance from it (106).

The observed phenomena, however, do not necessarily lead to this conclusion. According to Jakoby, the increased fibrinolytic enzyme activity found in the blood in phosphorus poisoning destroys the fibrinogen. Therefore, the formation of fibrinogen is not necessarily impaired, but its destruction could be accelerated. We do not know the

origin of the fibrinolytic enzyme; it could be an autolytic enzyme from the liver. It is not at all improbable that the enzyme is present in normal blood and that in certain circumstances there is a lack of normal inhibitors which otherwise might hold the fibrinolytic activity in check. In summary, observations on the blood in phosphorus poisoning have not yielded conclusive information concerning the origin of fibrinogen.

Another method for removing fibrinogen from the blood has been presented by Dastre (96). He removed blood from a dog, defibrinated it, and reinjected it intravenously. After this had been repeated a number of times there was no detectable fibrinogen left in the blood which consequently became incoagulable. This situation, however, prevailed only for a short time. After 24 to 48 hours the fibrinogen re-appeared and often in greater concentration than before the experiment. Mathews confirmed this observation and attempted to find by removing various organs from these afibrinogenemic animals those which were important for the reproduction of fibrinogen (141). He found that only by extirpation of the entire intestinal tract was the re-appearance of fibrinogen materially hindered. This led him to conclude that the most important source of fibrinogen was the intestinal tract. Incidentally, the production of fibrinogen still occurs during starvation.

In confirmation of the findings of Pavlov and Bohr (402), Mathews found that if the entire contents of the peritoneal cavity were excluded from the circulation, the blood in the anterior part of the animal became very hypocoagulable. However, this defect was not the result of a lack of fibrinogen. (Furthermore, Bohr's observations were seriously questioned by Contejean [311].)

Mathew's conclusion concerning the importance of the intestinal tract is not entirely convincing since such radi-

cal extirpation constitutes a stress so severe that the function of other organs may also be seriously impaired. In addition the animals survived but a short time. It should also be mentioned that according to Falk no fibrinogen can be found in the capillary blood of cadavers (108). This observation is thirty-five years old and requires confirmation. The preceding account shows that investigations on blood which had been artificially rendered fibrinogen-free although interesting, did not yield reliable information concerning the site of formation of fibrinogen.

Other authors experimented with the perfusion of various organs. Nasse stated that perfusion of an extremity of a dog with serum yielded a coagulable fluid (8). Mathews, on the other hand, was unable to confirm this observation. My own experiments with perfusion of the liver yielded only negative results.

More reliable information resulted from investigations based on quantitative analyses of fibrinogen in various conditions. Numerous analyses date back to the days of humoral pathology. The investigations of Simon, Lehmann, Bacquerel and Rodier, Andral and Gavarret have been cited in Wunderlich's book (40). These experiments showed that the fibrin content of blood was variable but duplicate determinations usually checked within 2 to 5 per cent. Some diseases, especially croupous pneumonia and acute polyarthritis, were accompanied by a considerable increase in the fibrinogen concentration. Andral and Gavarret found as much as 1 per cent fibrin in the blood in one case of pneumonia.

These older investigations and more recent ones such as those of Dastre (96-100) and Pfeiffer (145-147) must be interpreted with considerable caution in any attempt to determine the origin of fibrinogen since they were based on fibrin analyses rather than on quantitative determina-

tions of fibrinogen. Hammarsten pointed out that all of the fibrinogen is not necessarily converted to fibrin, but that the amount of fibrin also depends on many other factors.

For this reason the studies of Dastre (100) concerning the site of the formation of fibrin are not entirely convincing. He took quantitative fibrin analyses in arterial and venous blood as his point of departure. He found, in agreement with earlier observers whom he cited (Lehmann, Brown-Séquard, C. Bernard and Simon), that the blood of the mesenteric vein contained much more fibrin than the corresponding arterial blood. This observation was later confirmed by Mathews (141). In agreement with Mathews' earlier opinion Dastre concluded that the intestine was the main site of fibrinogen formation. According to Dastre, the lung and the skin were also capable of producing fibrinogen under certain circumstances. However, his data were not particularly convincing, nor has his thesis concerning the destruction of fibrinogen in the liver been completely proved.

Most of the more recent studies suggest a relationship between the white blood cells and fibrinogen. Even in earlier studies there were interesting references to an increase in fibrin in those diseases which are accompanied by leucocytosis while the increase did not occur in abdominal typhoid, or at any rate was negligible. These observations were in essence confirmed by Pfeiffer (145). He distinguished two groups of diseases. Typhoid, malaria, sepsis, and nephritis occurred without an increase in the fibrin concentration, while pneumonia, polyarthritis, etc. (i.e., infectious disease which are accompanied by leukocytosis), showed an increase. Leukemia, on the other hand, did not show an appreciable increase in fibrin (146). Pfeiffer attributed this to the functional derangement of the leu-

cocytes in leukemics. Samson-Himmelstjerna previously attributed the frequently prolonged coagulation of leukemic blood to the same derangement in the leucocytes (86).

Recent investigations are more conclusive since they were no longer based on quantitative analysis of fibrin but rather on that of fibrinogen. A method for quantitative fibrinogen analysis was devised and standardized by Reye (150). Based on a suggestion by Hofmeister, Reye used ammonium sulfate in the separation of fibrinogen from other globulins. He found that the range of precipitation of fibrinogen in plasma diluted five times approximated between 13 and 28 per cent saturation with ammonium sulfate. The precipitation of the other globulins first began at 29 per cent. That this procedure guaranteed a sufficiently precise separation from the serum globulins was confirmed by Schwalbe (11) and others. Saturated solutions of sodium sulfate can also be used advantageously for the isolation of fibrinogen.

Reye's method, as later experiments proved, led to considerable progress since fibrinogen analysis by means of coagulation did not produce satisfactory results because the fibrinogen did not necessarily coagulate completely as was stressed by Hayem (125) in discussing the observation of Arthus (92). The salt precipitation method was used in quantitative fibrinogen analyses by Lewinski (136), Langstein and Mayer (135), and more recently by P. T. Mueller (144). Langstein and Mayer found a considerable increase in fibrinogen in pneumococcal and streptococcal infections (135).

The above observations emphasized the close relationship between leucocytes and lymphatic tissue on the one hand and the formation of fibrinogen on the other. Mathews stated dogmatically that the fibrinogen concentration

was a measure of the rate of disintegration of the leucocytes in the body. This statement is scarcely acceptable if only the circulating leucocytes are considered since Pfeiffer had already pointed out that not all instances of leucocytosis are accompanied by an increase in the fibrinogen concentration of blood (145). Mueller's studies, on the other hand, made it appear very probable that fibrinogen may be formed in the lymphoid as well as in the myeloid tissue (144). He examined the fibrinogen concentration in the blood and in the bone marrow of animals which had been injected with various pathogenic agents. He found only a modest increase of fibrinogen in the blood, but discovered that quite often the fibrinogen content of the marrow had increased excessively—indeed, occasionally to many times its normal value. This increase was so great that it could not be explained simply by the varying blood or lymph content of the marrow. Mueller's findings (144) are very convincing. The only qualification that might be entertained is that one could not be absolutely certain of the complete absence of other proteins in the "fibrinogen fraction" of the marrow. However, this is not an important point since after coagulation by blood serum, bone marrow aspirate did not contain proteins which precipitated within the range of the fibrinogen fraction. Mueller's studies actually represented an important step forward since they strongly implicated the bone marrow and lymphoid tissue as sources of fibrinogen. Thus he furnished a splendid explanation for the frequent, simultaneous occurrence of leucocytosis and of an increase in fibrinogen since both conditions may be related to stimulation of the blood-forming organs.

The second phase of coagulation consists of the transformation of fibrinogen into fibrin. Unfortunately, the mechanisms and chemical processes involved in this trans-

formation remain unexplained. The early theory of Alexander Schmidt that fibrin resulted from a synthesis of fibrinogen and the "fibrinoplastic substance" had been disproved by the research of Hammarsten. The latter also demonstrated that fibrin is not a calcium salt of fibrinogen as Arthus and Pekelharing had assumed.

There remain only two theories to be discussed concerning the change in fibrinogen during its conversion to fibrin. Some investigators thought that this involved a hydrolytic splitting of fibrinogen into fibrin and a soluble globulin which was called "fibrinoglobulin" by Hammarsten who first described it (122). Apparently this globulin was regularly found in pure fibrinogen solutions following coagulation and could also be found in blood serum, though in very small amounts. It was supposedly readily distinguished from serum globulin because it was precipitated more easily by neutral salts (a characteristic similar to that of fibrinogen), and it had a lower temperature of "heat-coagulation" (65° to 66°C).

The significance of this protein has not been definitely established. Because fibrinogen and "fibrinoglobulin" were precipitated under similar circumstances it was not possible to determine with certainty whether "fibrinoglobulin" existed in the fibrinogen solution as an impurity prior to coagulation or whether it originated from fibrinogen during coagulation.

Originally Hammarsten favored the belief that during coagulation there was a hydrolytic separation of fibrinogen into fibrin and "fibrinoglobulin." This assumption was supported by his observation that the concentration of fibrin in coagulated fluid was always less than the original fibrinogen concentration. However, Hammarsten later abandoned this theory of hydrolytic separation. He found that 100 per cent fibrinogen yielded greatly varying amounts of fibrin whose percentages ranged between 61

and 94. This, of course, rendered a hydrolytic process highly improbable. Hammarsten then assumed that "fibrinoglobulin" was nothing but a portion of the formed fibrin which corresponded to the previously mentioned "soluble fibrin" and was present in varying proportions depending upon the amount of anticoagulants present in the solution.

Hammarsten's earlier theory was later energetically defended by Schmiedeberg (152) and Heubner (127). Heubner attempted to prove that no more than half of the available fibrinogen was converted into fibrin during coagulation. Hammarsten's variable results were explained by the assumption that his fibrin was not pure and contained impurities which could be removed by a very dilute ammonia solution. Heubner considered Hammarsten's variable results no reason to abandon the attractive theory of hydrolytic splitting of fibrinogen since his own analyses of the elements of fibrinogen, fibrin and "fibrinoglobulin" could be very easily reconciled with this theory. However, this did not fully settle the question. In a more recent study Huiskamp (131) showed that "fibrinoglobulin" need not necessarily be of importance for coagulation since using sodium fluoride precipitation it was possible to obtain fibrinogen solutions which did not yield "fibrinoglobulin" during physiological coagulation or upon "heat-coagulation." He concluded that "fibrinoglobulin" did not originate during coagulation, but already existed in the fibrinogen solution, either as a simple contaminant or more probably in some loose bond with fibrinogen.

Moreover, all of the facts assembled to date do not make it imperative to assume a splitting of fibrinogen during coagulation. A better assumption is that fibrin originates from fibrinogen by an intra-molecular shift, and that a portion of the formed fibrin remains in solution. In agreement with Duclaux (3) this could be viewed as a process

resembling "heat-coagulation." Indeed, Hammarsten, whose best efforts were dedicated to research on the second phase of coagulation, considered this the more probable explanation.

Fick (192) questioned the entire concept that the coagulation enzymes had to combine with each molecule of the coagulation substrate (fibrinogen) in the same manner that other hydrolytic enzymes combine with each molecule of the substrate (e.g., casein). Rather he thought that once a fibrinogen molecule was converted to fibrin by thrombin it influenced neighboring unaltered molecules producing an instantaneous gelling of the entire solution by a contact-like effect without any further participation of thrombin. Latschenberger (204) and Walther (214), however, showed that this theory did not tally with all the known facts. The best working hypothesis is that every fibrinogen molecule establishes contact with thrombin even though the manner in which fibrinogen is transformed during coagulation has not been ascertained.

The characteristics of fibrin, the end-product of the transformation of fibrinogen, are so well-known that there is no need to stress any but the main features. During spontaneous coagulation fibrin is laid down in the form of a fragile mesh which encloses the blood cells and seemingly changes the blood into a solid mass. When blood is stirred during coagulation the fibrin is obtained in the form of tough, elastic, white fibrous masses which contain only a few red cells. When coagulation occurs slowly, giving the blood cells time to sink to the bottom, a poorly cellular fibrin mass forms in the supernatent. This type of fibrin clot has been called a "chickenfat clot" or "crusta inflammatoria" and was considered of great importance in humoral pathology.

The retraction of fibrin clots is a very interesting phenomenon. Following coagulation the blood clot consisting

of the fibrin network and the blood cells, sooner or later separates from the walls of the container and shrinks, squeezing out a clear, yellowish liquid, the blood serum. This phenomenon of retraction has not been sufficiently studied. It was formerly attributed to the elasticity of the fibrin network. This explanation is apparently incomplete since retraction occurs only when there are blood cells in the fibrin-network but not following the coagulation of cell-free plasma. Alexander Schmidt made this observation but attributed it to physical alteration in the fibrin which in turn he blamed on the delayed coagulation of cell-free plasma (54). However, not all of the observed phenomena could be explained by this interpretation. Fuld (193), Delezenne (410), and Spangaro (388) found that avian plasma coagulated very rapidly upon the addition of tissue juice but the clot did not retract. Fuld pointed out that cell-free mammalian plasma behaved similarly. These findings certainly suggested that the formed elements were as important for clot retraction as the elasticity of the fibrin. It is possible that the morphological elements augmented the elasticity of fibrin in a purely mechanical fashion, or that they were involved in some chemical process, possibly a weak fibrinolysis. However, the manner in which they influence clot retraction remains unknown. In some situations clot retraction did not occur despite the presence of blood cells. Hayem showed that this was the case in some forms of severe anemia and hemorrhagic diathesis, but even in such cases retraction was not always absent.

Fibrin has the peculiar property of absorbing enzymes (e.g., *thrombin) in rather large quantities. The absorp*tion of enzymes may lead to the phenomenon which has been referred to as "fibrinolysis." In reference to its solubility fibrin behaves similar to other precipitated proteins in that it is insoluble in water, and dissolves only very slowly in 1 per cent hydrochloric acid and sodium hydrox-

ide solutions. Most neutral salt solutions whether dilute or concentrated dissolve fibrin faster than acid or alkali do. Serum likewise dissolves formed fibrin at a variable rate. This can often be observed in the blood of birds and frogs and not infrequently in mammalian blood. Fibrinolysis in the blood is very apparent in experimental phosphorus poisoning as long as the blood is still coagulable. In such cases Jakoby found that the complete dissolution of a large clot took place in but a few hours (132). Even when added to normal blood, the serum in phosphorus poisoning showed marked fibrinolytic activity. Dastre demonstrated that fibrinolysis occurred in normal blood and varied considerably in intensity but often amounted to 8 per cent in 18 hours, enough to be a potential source of considerable error in the quantitative analysis of fibrin (98). The studies of Dastre and of Arthus and Huber (218) showed that fibrinolysis could not be explained by the effect of bacteria since it also occurred in sterile blood. It is logical to assume that this phenomenon resulted from the effect of a fibrinolytic enzyme. This idea is supported by the variations in intensity of the phenomenon.

The occurrence of fibrinolysis in salt solutions of blood is somewhat more difficult to explain. First it must be noted that fibrin from different species of animals does not react in the same manner with various solvents as demonstrated by Fermi (110). Denis pointed out that in one and the same animal the solubility of fibrin varied with the vessel from which the blood was removed (102, 103). Fibrinolysis also occurred with greatly varying intensity in salt solutions of plasma as well as of whole blood. It proceeded much faster at 40°C than at lower temperatures, a finding which does not necessarily establish its enzymatic nature. A striking finding was that fibrinolysis often occurred more rapidly in strong salt solutions (e.g., a 10 to 15 per cent solution of sodium chloride) than in

weaker ones. The enzymatic nature of the process appears confirmed by some observations by Plósz (148). I myself observed an extraordinarily intense fibrinolysis in experiments on fibrinogen solutions in which marked disintegration of the blood cells had occurred. Fibrinolysis was stronger in fibrinogen solutions than in whole plasma. Whether the salt concentration was the critical factor or whether anti-enzymes or other inhibitors were removed during the isolation of the fibrinogen, remains unanswered.

Not all investigators believe that the dissolution of fibrin in salt solutions is an enzymatic process. Limbourg (137), in particular, argued against this idea, and Salkowski (151) attributed the digestion of the fibrin to bacterial enzymes. Salkowski's idea at best probably applies only to a limited number of the recorded cases. Finally, Denys and de Marbaix (104) found that the dissolution of fibrin was augmented by the addition of chloroform, ether, alcohol, and similar substances. They ascribed a direct enzymatic effect to these substances.

Although fibrinolysis is not completely understood the preceding paragraphs indicate that many observations (particularly those of Dastre [98]) can best be explained by assuming the existence of a fibrinolytic enzyme. Green (116), Halliburton (197), and Dastre (98) believed that fibrinolysis resulted in the formation of two globulins, one of which might possibly originate from the trapped leucocytes or other substances emeshed in the clot. The two globulins were allegedly distinguished chiefly by their different solubilities, and could not be re-converted to fibrin by the addition of thrombin. According to Halliburton (197) their temperatures of "heat-coagulation" varied between 60° and 75°C depending upon the salt solution used.

Chapter VI

ANTICOAGULANTS (PEPTONE AND SIMILAR SUBSTANCES; LEECH EXTRACT AND OTHER ANTITHROMBINS)

THE PRECEDING chapters have shown that the coagulation of blood is a highly complicated enzymatic process. The situation is further complicated by the effects of anticoagulant substances which undoubtedly also play a role in normal coagulation. Their significance has, however, not been established. Therefore, it seems advisable to document the effects of artificial anticoagulants, or "thrombases," as Duclaux called these substances (3). Such an investigation should yield important clues concerning the antithrombin of blood and the coagulation process itself.

Coagulation depends on the collaboration of many different substances—at least two precursors of thrombin as well as soluble calcium salts and fibrinogen. Therefore, the inhibition of coagulation can be brought about in many different ways, depending primarily on the site of action of the particular anticoagulant. It is incumbent upon the investigator to ascertain the cause of impaired coagulation in each instance with the greatest possible accuracy.

Some anticoagulant substances have already been mentioned briefly in the preceding pages. It is an old observation that neutral salts in appropriate concentration prevent coagulation. Alexander Schmidt and, more recently,

Bordet and Gengou (185) showed that neutral salts inhibited particularly the production of thrombin from its precursors, and in stronger concentration also suppressed the effect of the formed thrombin.

Oxalates, fluorides, citrates and soaps (i.e., salts which neutralize calcium salts) act in a different manner and require much smaller concentrations. As demonstrated earlier their anticoagulant activity also depends upon preventing the formation of thrombin. The clot-inhibitory effect of bile salts which von Samson (85) and Nauck (81) investigated may be based on a similar principle. The cooling of plasma which Alexander Schmidt frequently and successfully employed to prolong coagulation, is primarily based upon retardation of thrombin production. This was confirmed by the results of Bordet and Gengou.

There are many other ways of inhibiting coagulation. The addition of alkali, acid, sugar solution, or a large volume of water inhibits coagulation (Stodel [446]). When the carbonic acid concentration of the blood is high, clotting likewise is impaired. It is not always possible to elucidate the cause of the inhibitory action. At present such measures are not of very great theoretical significance.

Observations supporting the existence of clot-inhibitors in a more specific sense of the word are much more important for the theory of coagulation. According to Arthus (1) the numerous anticoagulants of this type could be divided into two groups. The first group consisted of those which had no anticoagulant effect per se but only brought about the development of inhibitors in the body through the mediation of intact organs. The prototype of this group is peptone. Various organ extracts also belong in this category. The second group included anticoagulants which were active without assistance from the body, and thus were effective *in vitro* as well as *in vivo*. These were leech

extract, cobra venom, "histone," and "cytoglobin." The last two proteins have already been discussed. No specific information is available concerning their activity. The anticoagulants of blood and tissue also belong in the second group although little is known about them.

SECTION 1: *The Effect of Intravascular Injections of Peptone*

Schmidt-Muehlheim, working in Ludwig's laboratory, found that under certain circumstances intravenous injections of Witte's peptone rendered the blood of dogs incoagulable (285). At about the same time a similar discovery was made by Albertoni (217). This interesting observation was soon confirmed and extended by Fano (256). The literature on peptone blood has since grown enormously, especially through the efforts of the French workers. At present this is the most thoroughly investigated field of blood coagulation.

In order to render blood incoagulable about 0.3 gm of peptone per kg weight of the dog had to be injected. Furthermore, the injection had to be given very quickly and the dog to be fasting. When the same amount of peptone was injected slowly or when the animal was not fasting, the result was no more than a prolongation of coagulation. It was also necessary to use the intravenous route of injection. Contejean (238) found that intraperitoneal injections of much larger amounts had no effect.

Not all dogs reacted similarly to the injection. In some animals the previously mentioned amount only produced a more or less insignificant prolongation of coagulation. Indeed, according to Thompson (291), some dogs demonstrated such a great resistance that even a dose of 0.5 gm per kg had no anticoagulant effect. However, Gley (263) showed that this resistance is strictly a relative one since very large amounts retarded coagulation in all dogs.

Cats reacted to peptone injections in a manner similar to dogs. Rabbits and guinea pigs were extraordinarily resistant, a fact already known to earlier investigators. Gley (265) and more recently, Persano (282) found that much larger amounts of peptone, often more than 1 gm/kg, were needed to retard coagulation appreciably in these animals.

The tremendously variable reactions of different animals emphasize that the peptone effect is not as simple as that of oxalate and other anticoagulant salts. The failure of the blood to coagulate after peptone injections is not due to peptone per se. Participation of the body is necessary. *In vitro* much larger doses of peptone were required to produce an anticoagulant effect than *in vivo*, a fact which was known to an earlier generation of investigators as shown by the observations of Shore (286), Dastre and Floresco (245), Camus and Gley (229), and Gley (265). All of these observers agreed that the amount of peptone needed to produce an anticoagulant effect *in vitro* was ten to fifteen times greater than that needed on injection into the body. There must therefore exist in peptone a substance which on reaction with the body hampers coagulation in some manner.

Witte's peptone is by no means a uniform substance. It is largely composed of a mixture of albumoses and peptones. Therefore, it was important to attempt to isolate the active component. The findings of Schmidt-Muehlheim (285) and Fano (256) suggested that the active substance was formed during peptic digestion of proteins. On the other hand, "tryptone," the product of pancreatic digestion, did not influence coagulation. Pick and Spiro (283) subsequently confirmed this observation. Arthus and Huber (218), however, produced anticoagulant gelatoses and caseoses by tryptic digestion of gelatin and casein. The contradictory results obtained may have been due to different intensity of the tryptic digestion. Since the al-

bumoses of Arthus and Huber had only a slight inhibitory effect, we may assume that trypsin destroyed the active component. Albumoses produced by hydrochloric acid digestion without pepsin had an anticoagulant effect as shown by Pick and Spiro, but those produced by digestion with alkali were ineffective. It is conceivable that the active component was destroyed by the alkali. Except for this it appears rather stable and is heat-resistant.

Attempts to ascribe the observed effect to a definite fraction of the albumoses have not yielded uniform results. It is difficult to compare the experiments of different workers since they were undertaken with diverse methods, and a uniform nomenclature was not employed. Pollitzer* thought he found the active substance in a "hetero-albumose" fraction, while Grosjean (276) focused attention on his so-called "propeptone" which was effective in much smaller amounts than the Witte peptone. The more recent tendency has been to disregard the albumose fractions and to implicate some impurity as the cause of the anticoagulant effect of peptone. Recently, however, Zaleski (294) has attributed the anticoagulant effect to the albumoses, especially the so-called "hemi-albumose." The interesting studies of Fiquet (261) and Thompson (291), on the other hand, indicated that the effect progressively decreased and finally vanished completely upon careful purification of the proteoses. Pick and Spiro (283) believed that the anticoagulant effect could not be attributed to any appreciable degree to various albumose fractions. They also felt that it could be inactivated by alcohol which coagulated the albumoses. Pick and Spiro concluded that the active component was only a contaminant which they called "peptozyme." This term will henceforth be used in

* Pollitzer, S.: On the physiological action of peptones and albumoses. *J. Physiol.* 7:283, 1886.

this paper. This may not be entirely accurate since because of its heat stability, peptozyme can hardly be termed an enzyme.

As previously stated, peptozyme is not an anticoagulant per se since *in vitro* it was almost totally ineffective. It brought about incoagulability only by a reaction with the body. Therefore, the mechanism of the peptone effect is very complicated. Information concerning it is still most readily obtained by investigation of the incoagulable peptone blood itself.

These investigations have not yet yielded completely satisfactory results. This is partly because of the complexity of the matter, but also because of the fact that the properties of peptone plasma are not entirely constant. These properties vary with the intensity of the peptone intoxication, the nutritional status of the experimental animal, and many other factors.

In many instances peptone blood clotted spontaneously sooner or later. As Fano (256) found it could always be coagulated by neutralization by acid, or the addition of water, a stream of carbon dioxide, or neutral salts, i.e., by many different processes. Fano claimed that plasma behaved differently than blood since the more it was freed of formed elements, the less readily plasma was clotted by the above procedures. Athanasiu and Carvallo (223) made the same observation. Wooldridge's observations suggested that cell-free peptone plasma could be coagulated as readily as peptone blood by dilution, carbonic acid, etc., unless it had been previously freed of Wooldridge's granular material which was precipitated at about 0°C. This substance which Wooldridge called "fibrinogen-A" was previously discussed in conjunction with his theory of coagulation. After removal of this substance (which could then no longer be considered an intact formed ele-

ment) peptone blood could no longer be coagulated by any of the above procedures. However, the addition of "tissue fibrinogen" brought about the formation of fibrin. Wooldridge considered that "tissue fibrinogen" had the same effect as "fibrinogen-A" and therefore could combine with "fibrinogen-B" (the fibrinogen of other workers) to form fibrin. According to Wooldridge thrombin did not clot peptone plasma. Fano (256) also concluded that thrombin was not always capable of coagulating peptone blood in contrast to the findings of Schmidt-Muehlheim (285).

It is easy to see that this is a very complex situation. There is no wonder that attempts to explain the peculiarities of peptone plasma have yielded varying results.

Pekelharing (170, 210) followed Wooldridge in starting with the assumption that a clot-accelerating substance, the so-called "fibrinogen-A," was precipitated from peptone plasma upon cooling. As stated earlier this substance was, according to Pekelharing, a nucleoprotein and the precursor of thrombin. It yielded thrombin on the addition of calcium salts. When this substance was removed, plasma no longer clotted spontaneously even though it contained fibrinogen. By contrast the addition of thrombin clotted peptone plasma normally as observed by Pekelharing (210) in opposition to the views of Wooldridge (454) and Fàno (256). The negative findings of the latter two investigators can be explained by the impotency of the thrombin solutions they employed. According to Pekelharing peptone plasma remained fluid because it was free of thrombin and only contained the precursor of the latter. Why is it that the precursor did not spontaneously convert to thrombin but only after certain procedures such as dilution and neutralization? Pekelharing believed that this was due to the fact that the albumoses bound the calcium salts loosely. He pointed out that the injection of albumoses produced a picture of intoxication similar to that following

the injection of calcium-precipitating salts, such as oxalates and soaps. This was further supported by his observation that the simultaneous injection of calcium salts and peptone did not render the blood incoagulable.

The views of Pekelharing are untenable. If the situation were so simple, the albumoses would have to have the same effect *in vitro* as *in vivo*. Furthermore, Arthus (1) pointed out that the different reactions of the dog and rabbit could not be explained by Pekelharing's simple hypothesis.

There were also objections seemingly less justified to the first of Pekelharing's theories. Dastre and Floresco (245) in particular, felt that while peptone plasma did not contain prothrombin, it did contain free thrombin since peptone plasma clotted equine pericardial fluid which contains no thrombin. That thrombin did not clot peptone plasma was attributed to the absence of certain basic substances necessary for coagulation. Dastre and Floresco (245) attributed the incoagulability of peptone plasma to an increase in alkalinity. This was by no means adequately proved. Athanasiu and Carvallo (224) opposed this viewpoint, asserting that peptone plasma could not possibly contain thrombin since it clotted regularly upon the addition of thrombin. Above all, a very potent thrombin solution had to be used since peptone plasma contained varying amounts of an antithrombin. The quantity of the antithrombin apparently depended upon the intensity of the peptone intoxication. Schmidt-Muehlheim (285), Grosjean (276), and Ledoux (325) had already noted the presence of this heat-labile anticoagulant. Subsequent investigators studied the origin of this substance very thoroughly. This will be discussed later. It is my personal opinion that not all the peculiarities of peptone plasma can be explained by the discovery of an anticoagulant.

This was clearly shown by the more recent studies by

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cleoproteins and thrombokinase as was previously mentioned. After the removal of the precipitate peptone plasma (which still contained prothrombin) no longer coagulated except upon the addition of thrombokinase. It was no longer coagulated by dilution or other procedures. Calcium salts were also found in peptone plasma. To date there is no proof that they are either bound or present in a non-ionized form.

It is clear that peptone plasma contains all the known factors needed for the production of thrombin. Nevertheless, thrombin formation was absent. As yet there is no information concerning the failure of these substances to react. This observation is reminiscent of the statement by Bordet and Gengou (185) concerning the reaction of cell-free plasma in paraffined containers. Under those circumstances all the factors necessary for coagulation were also present, but thrombin formation was absent until the plasma was placed in contact with a wettable surface.

Be that as it may, it appears certain that there are two reasons for the incoagulability of peptone blood. The important factor is the absence of thrombin formation for unknown reasons. In addition the presence of an anticoagulant is also significant. According to the concurring observations of Fuld and Spiro (195) and Morawitz (328) this anticoagulant should be considered an antithrombin and not an antithrombokinase. It is naturally unsatisfactory to assume the presence of two independent anticoagulants. However, this cannot be avoided. It is quite possible that a simple connection exists between these two phenomena. It must be stressed that the inhibitor must result from the action of "peptozyme" and not from an antithrombin normally present in blood. This can be concluded from the numerous interesting studies of French scientists which concerned the origin of this antithrombin.

The first attempt to find the site of the formation of

Fuld and Spiro (195) and by my own investigations (328). In these studies the investigators' ideas concerning the origin of thrombin from several substances were applied to the problem of peptone plasma. These experiments demonstrated first of all that the peptone plasma undoubtedly contains an anticoagulant in varying amounts. These investigators believed that this substance was obviously a product of the body and originated in all probability through the effect of the "peptozyme" upon the organs of the body. The anticoagulant was considered an antithrombin since it could neutralize a certain amount of thrombin. But how could some relatively mild procedures which seemed hardly capable of injuring the rather durable inhibitor bring about coagulation in peptone plasma? The presence of an inhibitor alone did not suffice to explain this phenomenon. These investigators relied on Pekelharing's explanation that the formation of thrombin did not take place in peptone plasma (170). They thought that peptone plasma actually contained no thrombin. This idea was apparently proved by the fact that decalcified peptone plasma was not affected by any of the procedures which brought about coagulation of peptone plasma that was not decalcified.

What was the reason for the absence of thrombin? Does peptone plasma lack any of the three factors necessary for the formation of active thrombin? Prothrombin was undoubtedly present since the plasma coagulated readily upon the addition of thrombokinase, i.e., tissue juice. This had been proved by the observations of Wooldridge (454), Hewlett (201), and many others. Was thrombokinase perhaps lacking? This could not be demonstrated since otherwise completely acellular and platelet-free plasma would not be coagulated by neutralization or dilution. In fact the presence of thrombokinase in peptone plasma could be proved. The precipitate obtained at 0°C contained nu-

This assumption was further supported by the observations of several investigators (e.g., Spiro and Ellinger [289]) that the lymph of the thoracic duct was the first to become incoagulable after peptone injections. The previously described investigations, however, gave no information as to what actually occurred in the liver.

This was a question to which Delezenne dedicated his efforts (250). He found that perfusion of the liver following peptone injections yielded an anticoagulant in the perfusate. This perfusate was capable of prolonging or completely inhibiting the coagulation of normal blood *in vitro*. Ten or twenty drops of the perfusate sufficed to keep 10 cc of blood in a liquid state for many hours or indeed for days. The intensity of the anticoagulant effect was extremely variable and depended upon many factors. The liver of starved dogs yielded a more effective anticoagulant solution than that of dogs which were killed in a post-absorptive state. The first portions of the perfusate seem to have more activity than those removed later. This apparently indicated that the ability of the liver to produce the anticoagulant was limited. Perfusion of other organs never yielded an anticoagulant. The anticoagulant not only affected the coagulation of canine blood but also that of other animals.

What mechanism brought about the production of this anticoagulant? Delezenne (252, 253) thought that the leucocytes played the most important role. He chose as his point of departure the observation of von Samson (85) that peptone injections resulted in a marked, temporary leucopenia. This is an established fact which has been repeatedly confirmed by such workers as Wright (293) and Athanasiu and Carvallo (219). Delezenne assumed that this not only concerned an altered distribution but also an actual disintegration of the leucocytes. He thought that this

the antithrombin was undertaken by Contejean (236). He carried out numerous experiments involving extirpation of various organs and concluded that following peptone injections, the liver and perhaps also the intestine were chiefly responsible for the formation of the antithrombin. At any rate, they played a significant role in the origin of the anticoagulant effect, even though as mentioned previously this was not necessarily considered synonymous with the formation of an antithrombin. Contejean did not assign the role to the liver exclusively but thought that all cells participated in the process to a greater or lesser degree. Other investigators were of the opinion that the liver was solely responsible for the incoagulability of the blood after the peptone injection. It must be admitted that the uniform results obtained by Gley (266, 268, 269), Gley and Pachon (262, 264, 267), and Hédon and Delezenne (277) *provided compelling proof for this hypothesis.* Gley and Gley and Pachon either excluded the liver from the circulation or damaged it in many different ways and under such circumstances found that the peptone effect was always absent. This occurred in destruction of the liver by the injection of acids into the ductus choledochus, by extirpation of the liver, and by ligation of the lymphatic vessels of the liver. The last observation was not confirmed by Starling (290) and Delezenne (250). Hédon and Delezenne indicated that the peptone effect was absent after the creation of an Eck fistula. Even the nervous system of the liver seemed to take part. According to Contejean (237) and Gley and Pachon (267) extirpating the coeliac plexus or damaging it by the injection of cocaine inhibited or considerably reduced the anticoagulant effect of peptone injections.

These studies indicated with considerable certainty that the liver plays an important part in the peptozyme effect.

organ. However, as has been demonstrated there can be no doubt that peptone plasma contains thrombokinase, perhaps even in normal amounts. This deprives Delezenne's theory of part of its basis. Furthermore, "histone" has not been demonstrated in peptone plasma, and the properties of this plasma were not identical with those of the "histone" plasma of Lilienfeld (433), a fact which Arthus correctly stressed. Delezenne's studies, thus, have shown no more than that the antithrombin of peptone plasma originated in the liver, perhaps due to the participation of the leucocytes. No explanation exists for the absence of thrombin formation in peptone plasma even though the latter contains all of the necessary components for thrombin formation. It is clear that the mechanism of the peptone effect has not been satisfactorily explained.

There is room for a few remarks concerning the properties of the antithrombin. Its chemical nature remains unknown, but even such a simple problem as its heat-resistance has not been answered unequivocally. In a dry state it certainly tolerates temperatures as high as 120° to 140°C as the observations of Camus have shown (231). Even in solution the antithrombin is only slightly damaged by boiling according to Delezenne (250). On this basis Delezenne identified this substance with the anticoagulant of the leech. By contrast Fano claimed that the antithrombin is not heat-resistant (256). It is possible that the negative results of Fano could be explained by the adsorption of the antithrombin on the protein precipitate.

A peculiar, poorly understood phenomenon is the resistance to the action of peptozyme. A distinction must be made between acquired and natural resistance. Natural immunity is found to a certain extent in rabbits and in some dogs. Acquired resistance is regularly present

disintegration of white cells permitted anticoagulants as well as procoagulants to be released into the plasma. The clot-promoting substances (which he considered akin to Lilienfeld's "leuconuclein" [433]) were thought to be retained or inactivated by the liver. On the other hand, the anticoagulant, "histone," apparently passed the liver unaltered. Thus, peptone plasma was thought to have properties similar to those of the "histone" plasma of Lilienfeld. The individual experiments upon which Delezenne's theory was founded will not be discussed in further detail. Briefly, his ideas were largely based on the observation that only perfusates containing leucocytes yielded the anticoagulant, and on some observations on leucocytotoxic sera which like peptone inhibited coagulation. The interpretations of Delezenne were not entirely satisfactory. Arthus (1) presented data which could not be readily reconciled with Delezenne's theory. First of all there was no decisive proof that peptone injections produced a leucolysis. Many investigators, especially Athanasiu and Carvallo (221), and more recently Ruechel and Spitta (483), tried to demonstrate that the leucopenia was not due to disintegration of leucocytes but rather to an altered distribution of these cells, especially to an accumulation of white cells in the splanchnic region which was severely damaged by peptone injections with a concomitant fall in blood pressure. The striking mobility of the leucocytes in peptone blood which Fano (256) and Athanasiu and Carvallo emphasized certainly did not suggest that these cells were damaged. But even if the occurrence of leucolysis is assumed, Delezenne's investigations did not completely explain the peculiar properties of peptone plasma. According to Delezenne the "leuconuclein" (which corresponds to thrombokinase) was retained in the liver, and only the anticoagulant, "histone," passed through that

organ. However, as has been demonstrated there can be no doubt that peptone plasma contains thrombokinase, perhaps even in normal amounts. This deprives Delezenne's theory of part of its basis. Furthermore, "histone" has not been demonstrated in peptone plasma, and the properties of this plasma were not identical with those of the "histone" plasma of Lilienfeld (433), a fact which Arthus correctly stressed. Delezenne's studies, thus, have shown no more than that the antithrombin of peptone plasma originated in the liver, perhaps due to the participation of the leucocytes. No explanation exists for the absence of thrombin formation in peptone plasma even though the latter contains all of the necessary components for thrombin formation. It is clear that the mechanism of the peptone effect has not been satisfactorily explained.

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after the peptone effect has worn off, and usually persists for no more than 24 hours. Following a peptone injection the blood regains its coagulability usually in about 2 hours if a medium-sized dose is used but within the next 24 hours it is not possible to inhibit coagulation by another injection of peptone. Schmidt-Muehlheim (285) was familiar with this phenomenon of resistance. Although it has since been thoroughly investigated, a satisfactory explanation is still lacking. Fano (256) tried to explain this by an hypothesis which is reminiscent of Pasteur's theory of exhaustion. He theorized that the first injection exhausted the material necessary to produce the anticoagulant which was then re-formed only slowly. More recently Nolf expressed a similar opinion.

Most observers do not accept Fano's interpretation, chiefly because it was possible to induce resistance to peptozyme without a preceding stage of incoagulability. This apparently excluded the mechanism of exhaustion of the anticoagulant as a possibility. Gley and LeBas (273) maintained that very small amounts of peptone, which do not alter the coagulability of the blood, can invoke a phase of resistance against an otherwise effective amount. According to Fano, tryptone was capable of inducing a state of resistance against peptone injections without altering the coagulability of the blood. There were additional ways of immunizing dogs against subsequent peptone injections. Gley alluded to the protective effect of a preceding injection of rabbit serum, and Contejean (238) to the prior administration of peptone blood itself. These facts do not support Fano's theory of exhaustion.

The attempt to base the refractory state on changes in the leucocytes also yielded negative results. It might be presumed that the second injection could not bring about lysis of the leukocytes and that therefore, according to Delezenne's thesis, no more anticoagulant could be re-

leased. But this is not tenable either since Athanasiu and Carvallo (223) showed that the second injection of peptone which produced no anticoagulant effect still resulted in a leucopenia.

Finally, there remained the possibility that an antibody was formed against the anticoagulant of peptone blood. The situation could be compared to that of antitoxic immunity. Spiro and Ellinger (289) first emphasized this possibility after they rejected Fano's theory of exhaustion as improbable. The opinion of Spiro and Ellinger at present must be considered as the most acceptable.

In general, the peptone effect is still in need of explanation. Peptone plasma resembles in many respects goose plasma produced according to the method of Delezenne from which it is differentiated chiefly by the presence of thrombokinase in the former. As will be seen later an inhibitor was also found in the normal goose plasma although apparently not in such large amounts. The injection of peptone into birds resulted in the appearance of a very potent antithrombin according to Spangaro (287, 288), Fuld and Spiro (195), and the present author (208). Such goose plasma clotted just about as readily as normal plasma on the addition of tissue juice but much less readily when thrombin in the form of normal goose serum was added. This situation is in harmony with the observations made on mammals after peptone injections.

Translators' Addendum: Subsequently many workers* have presented evidence that the anticoagulant, heparin, is present in peptone blood, and that its presence is chiefly or totally responsible for the coagulation defect in peptone

* Howell, W. H: The purification of heparin and its presence in blood. *Am. J. Physiol.*, *71*:553, 1925.

Fuchs, H. J: Ueber die Beteiligung des Komplements bei der Blutgerinnung. *Arch. Exper. Path. u. Pharmacol*, *145*:108, 1929.

Quick, A. J: On the coagulation defect in peptone shock. *Am. J Physiol.*, *116*:535, 1936

blood. When one considers the many variables which may influence the anticoagulant activity of heparin*, the conflicting evidence presented by earlier workers is readily understood. Wilander† in addition has shown that extensive damage occurs to the hepatic mast cells of dogs during peptone shock, thus giving a likely explanation for the importance of the liver in releasing heparin to produce the coagulation defect in peptone shock.

SECTION 2: *The Anticoagulant Activity of Substances of the Peptone Group and the In Vivo Action of Tissue Juice*

Under the name of "peptone-like" substances Delezenne (315) grouped various agents which on injection had an anticoagulant effect similar to that of peptone but were inactive *in vitro*. The oldest known substance was the serum of *Murænidæ*.‡ Mosso (330-332) found that the intravenous injection of small amounts of eel serum was very toxic to dogs and in addition to other phenomena brought about a striking fall in blood pressure and prolonged incoagulability of the blood—findings similar to those brought about by peptone. Mosso attributed this to a heat-labile protein, "Ichthyotoxicum." Springfeld (342) confirmed the findings of Mosso, but Delezenne (315) was *the first to attempt clarification of the mechanism of* action. Eel serum was active in amounts of 0.01 to 0.03 cc but only on intravenous injection and not *in vitro* or by intraperitoneal injection. The mechanism of action was thought to resemble that of peptone since perfusion experiments apparently demonstrated that the liver under the influence of the eel serum and in conjunction with

* Hartmann, R. C.: A critique of the "heparin tolerance" test. *Bull. Ayer Clin. Lab.*, 4:43, 1952 (May).

† Wilander, O.: Studien ueber Heparin. *Skandinav. Arch. Physiol.*, 81: supple. 15, 1939.

‡ This refers to the family of Moray eels.

leucocytes produced an anticoagulant. The similarity to peptone extended to the finding that eel serum produced a state of incoagulability in dogs much more readily than in rabbits.

Delezenne (315) likewise studied the anticoagulant activity of various organ-extracts such as crab muscle and snail extract. Heidenhain (424) clarified the peculiar physiological action of these agents. Similar active substances were studied in lower animals by Abelous and Billard (295, 296), Camus (307), Camus and Lequeux (308), and Couvreur (313).

Most of these extracts had in common the property that they accelerated coagulation *in vitro* but on injection into the body rendered the blood incoagulable. Just as in the case of peptone it was shown that the liver was essential for the production of the anticoagulant. These agents also produced a marked leucopenia further supporting the analogy to peptone. The resemblance goes even farther. Delezenne showed that a previous injection of one of these substances protected the animal from an injection of peptone and vice versa.

All of this made it quite apparent that the action of peptone and these other substances was very similar. Do all of the substances of the peptone-group contain the same active substance, peptozyme? This was considered possible but did not necessarily follow since peptozyme was heat-stable, whereas the active substance in eel serum was destroyed by heat according to Mosso (330).

Experience has shown that chemically different substances possess the same ability to produce alterations in the body which bring about the inhibition of coagulation. This seems all the more plausible since not only different tissues but, according to Camus (309, 310), milk and various vegetable products (312, 322) may have an anticoagulant effect when injected into the vessels. According to

Albertoni (297), Salvioli (340), and Dastre and Floresco (314) similar properties are found in many soluble enzymes.

Does the blood in all these cases mimic peptone blood? According to Delezenne (314) it is possible. It has, however, not been proved. More recent observations on the *intravascular effect of organ extracts seem to indicate that* the incoagulable blood need not always have the exact properties of peptone blood.

That the injection of tissue juice, i.e., thrombokinase, frequently causes intravascular clotting has already been mentioned. This is obvious since thrombokinase in circulating blood interacts with calcium salts and prothrombin to form thrombin and therefore produces thromboses just as in extravascular plasma. The studies of Wooldridge (454), Groth (63), Wright (394), and others have shown *that the body undoubtedly possesses the ability to counteract a dangerous degree of thrombin formation in the* circulating blood. Coagulation does not always occur upon injection of thrombokinase. Often the thromboses are restricted to certain vascular areas, especially the portal system. Blood collected following such an injection remained liquid or coagulated only very slowly. In such circumstances thrombokinase injected into the circulation had exactly the opposite effect as *in vitro*. What are the reasons for the formation of thromboses on the one hand, and *the incoagulability of the blood on the other?* The extent and ease with which thromboses formed depended primarily upon the amount of thrombokinase injected and the type of animal used. According to Wooldridge and Pekelharing (210) it was much easier to produce intravascular thromboses in rabbits than in dogs. Small amounts of tissue juice produced only a more or less strong negative phase. Certain other factors, e.g., the

CO_2 content of the blood, also augmented the occurrence of thromboses, as Wright's experiments with asphyxiated and apneic animals have shown (393). For that reason thromboses were supposed to occur most frequently in the blood of the portal system which is so to speak strikingly "venous" in character.

The occurrence of intravascular clotting after injections of thrombokinase can be readily understood. It is more difficult to explain the negative phase of the reaction. Pekelharing and Wright attributed the phenomenon to the separation of an albumose from the tissue nucleoprotein, and thereby made an analogy to peptone plasma. Since it has been shown that the albumoses did not directly cause the prolongation of clotting in peptone plasma, and since the incoagulable plasma was not regularly shown to contain albumoses (Halliburton and Brodie [371], Martin [378]), this explanation can be disregarded.

Does thrombokinase possibly have an effect similar to that of peptozyme, or in other words, does the incoagulable *plasma following the injection of kinase* ("kinase plasma") have properties like peptone plasma? This remains controversial. Wooldridge (454) apparently thought that the two plasmas were not identical since tissue juice coagulated peptone plasma readily but clotted "kinase plasma" only with great difficulty. Groth (63) made the same observation while Wright (395) ascribed the inhibition of coagulation in both plasmas to identical causes. The newer studies of Boggs (351) seem to indicate that such is not the case since "kinase plasma" did not coagulate after dilution with water, neutralization with acetic acid, or upon addition of calcium chloride. Upon addition of thrombin it coagulated, however, more readily than peptone plasma, whereas thrombokinase, in contrast to its effect on peptone plasma, was barely able to

make "kinase plasma" clot. Boggs, finally, confirmed an old observation of Wooldridge that "kinase plasma" coagulated upon the addition of peptone plasma. This apparently proved that "kinase plasma" and peptone plasma were not identical. The former does not contain, like the latter, an antithrombin, but possibly an antikinase. There is, however, too little known in this field to allow one to draw definite conclusions.

It is interesting that an injection of kinase (i.e., tissue juice) produced an immunity of sorts. This fact, too, was indicated in Bogg's confirmation of Wooldridge's earlier observations. Repeated injections of progressively increasing amounts of tissue juice in rapid succession did not produce intravascular thromboses in rabbits, even when eventually ten to twenty times the usually lethal amount was injected. The blood merely became completely incoagulable.

It is still too early to explain the cause of impaired coagulation following injection of tissue juice. One cannot even decide whether the inhibitor is produced by the body as in the case of peptone plasma or originates in the tissue juice itself. In addition to thrombokinase the tissues contain heat-resistant, anticoagulant substances according to Lilienfeld (433), Conradi (358), and Dastre and Floresco (361).

It is clear that the influence of the body on the coagulability of the blood is still far from being satisfactorily clarified. This remains a very complex matter. There undoubtedly exists a relationship between the blood and the parenchymatous organs which, under certain conditions, influences the coagulability of the blood by the production and release of anticoagulants. It remains undecided whether these substances are already present in the tissues or whether they are antibodies produced against

the different precursors of thrombin. Nor is any information available as to whether or how the organs under normal circumstances contribute toward maintaining the liquid state of blood as well as its coagulability.

In connection with the reaction of the blood following injections of tissue juice it should be noted that Halliburton and Pickering (372) obtained very similar results by injections of synthetic colloids produced by the method of Grimaux (369). These colloids were basically condensation products of different amino acids and certainly contained no thrombokinase since the way in which they were produced would guarantee the absence of the latter. Despite this the results were said to be quite similar to those brought about by the intravenous injection of tissue juice. They brought about either intravascular coagulation or a negative phase of coagulation. According to Pickering (384) *in vitro* these colloids had only an inhibiting effect. No explanation of this phenomenon has been forthcoming. Halliburton and Pickering intimated that injection of these substances, just like the injection of peptone or tissue juice, produced a leucopenia. However, they justifiably showed no inclination to ascribe the observed effects to the destruction of leucocytes.

SECTION 3: *Substances which act as Anticoagulants both In Vivo and In Vitro (Hirudin, Snake Venom, and the Antithrombins of Blood and Tissue)*

The preceding sections have dealt with substances which although not antithrombins per se can produce anticoagulants in the body. By contrast, a number of substances were known which inhibited coagulation *in vivo* and *in vitro* in a similar manner. It is clear that the action of the latter group can be much more readily explained than that of the former.

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lief was confirmed by Dickinson and later by Fuld and Spiro (195) and my own experiments (328). These studies left no doubt that hirudin could neutralize thrombin, and that it contained an antithrombin of approximately the same quantitative effect. One drop of leech extract, for example, neutralized the effect of 10 drops of blood serum but not that of 20 drops. Pekelharing (210), however, claimed that an additional factor was responsible for the anticoagulant effect of the leech extract. He thought that leech extract prevented the escape of a thrombin precursor (i.e., thrombokinase) from the formed elements. This may have been related to the observation that leech extract preserved the formed elements particularly well. This was invoked as the reason for the finding that cold-precipitable nucleoprotein, the precursor of thrombin, could not be demonstrated in leech extract plasma. When, however, the formed elements in incoagulable hirudin blood were destroyed by the addition of water, the precursor apparently entered the plasma and coagulation took place. Hirudin plasma, in contrast to peptone plasma, did not clot upon the addition of acetic acid or by dilution with water. Hirudin also did not bind calcium salts. The ability of hirudin to fix thrombokinase was not completely and satisfactorily demonstrated since under certain conditions it was possible to obtain a precipitate containing nucleoprotein from the hirudin plasma. This was, however, only of secondary importance as far as the function of hirudin was concerned. The properties of hirudin plasma can be very well explained by the assumption that the liberation of thrombokinase into the plasma and the formation of thrombin takes place anyway to a greater or lesser extent, whereas, the formed thrombin is neutralized by hirudin.

The *reaction of hirudin plasma to the addition of*

The oldest and best-known of these substances is hirudin, the anticoagulant of the leech. It has been known for a long time that the triangular wounds produced by therapeutic leech bites continue bleeding for a long time. This fact used to be ascribed to the shape of the wound, until Haycraft (324) in 1884 proved that leeches contain an anticoagulant. This apparently originates in the mouth glands of the leech and is thus found only in the anterior part of the animal. Minor effects in the posterior part of the body were ascribed to the presence of hirudin in the intestinal tract. Haycraft characterised hirudin as a heat-resistant, water-soluble substance which was excreted unchanged in the urine following injection into the body. It received its name from Jakoby and Franz (320). In Dickinson's method potent extracts were prepared by drying leech heads which had been preserved in alcohol (317). The heads were then pulverized and extracted with water for several hours, using 5 to 10 cc of water per head.

The isolation of hirudin in a pure state has often been attempted. The earlier experiments of Dickinson, Bock (300), and others did not lead to satisfactory results. Dickinson intimated that hirudin was a protein. Later, Franz (320) devised a successful method for isolating pure hirudin which was further refined by Bodong (301). The process was largely based on removal of the protein from the leech extract by chloroform extraction, heating, and subsequent dialysis. According to Franz, hirudin, which does not dialyze (or at best moderately so), is a secondary albumose.

Hirudin has both an intra- and extravascular anticoagulant effect. Haycraft speculated on the nature of the anticoagulant effect, believing hirudin contained an antithrombin which either united with thrombin to form an inactive compound or else destroyed the latter. This be-

All anticoagulants found in blood-sucking animals are probably of great significance for the nutrition of the animals and in their physiological behavior closely related to hirudin. The existence of such substances in ticks (ixodes ricinus [338]) and in anchylostomum caninum (326) is well-known. Undoubtedly most blood-suckers are provided with mechanisms designed to keep the blood liquid in the intestinal tract.

Lilienfeld's histone chloride (433) might also possibly be compared to hirudin. Since there is, however, no recent information concerning histone plasma, no definite statement can be made.

So far, discussion has been limited to anticoagulants which can be considered antithrombins in the narrower sense of the term (i.e., substances which can bind thrombin and possibly also prothrombin). There exists, however, a substance in some snake venoms which is undoubtedly an antikinase.

It is an old observation that the blood of animals which have been killed by snake bites sometimes does not clot. This was pointed out by Fontana (319) and later confirmed by Brainard (304), Weir-Mitchell (346), and Halford (323), who investigated various American and Australian snake venoms. These investigators pointed out that after an injection of snake venom prolonged coagulation occurred, particularly when the poison had the opportunity to mix extensively with the blood. On the other hand, animals which died immediately after the injection frequently showed multiple intravascular thromboses.

Heidenschild (66), Feoktistow (318), Martin (327), Stephens and Myers (343), and I paid particular attention to this problem. Heidenschild, a disciple of Alexander Schmidt, attributed the incoagulability of the blood after an injection of the venoms of Naja and Crotalus to a

thrombokinase has been investigated by the author (328). Hirudin plasma occasionally coagulated upon the addition of small amounts of tissue juice; in other instances it remained liquid even upon the addition of large amounts. This depended upon the amount of hirudin added. When little hirudin was present, the added thrombokinase formed so much thrombin from prothrombin that the antithrombic activity was completely overcome. On the other hand, even very large amounts of tissue juice did not produce enough thrombin to bring about coagulation when there was a large amount of hirudin in the plasma since the amount of prothrombin in plasma was not unlimited. This proved clearly, as did the experiments of Fuld and Spiro (195), that the relationship between thrombokinase and hirudin is not one of neutralization. Hirudin like the anticoagulants of peptone plasma, neutralized only thrombin and perhaps also prothrombin. Despite their similar action, the two antithrombins need not be identical in nature as Delezenne believed (250).

Recently Bodong (301) expressed the opinion that hirudin altered fibrinogen and thereby produced incoagulability of the plasma. The uniformly different results of all earlier investigators contradict this.

Ledoux (325) was unable to bring about resistance to the effect of leech extract. Some inkling of an acquired resistance was observed by Contejean (311). Subsequently, Wendelstadt (345) succeeded in demonstrating an acquired resistance. He was actually able to produce an antibody of the third class.

Finally it should be mentioned that according to Bosc and Delezenne (303), hirudin blood had a very striking resistance to bacterial action. These two scholars ascribed this to the release of alexines from the leucocytes into the plasma.

of cobra venom into the circulation resulted only in the negative phase. Intravascular thromboses were not found. Thus there is no reason to assume the formation of active substances by the body as was the case following peptone injections. The incoagulability of the blood can be readily explained by the presence of antikinase in cobra venom. The incoagulable plasma produced by the injection of cobra venom resembled more closely "kinase plasma" than peptone plasma. It coagulated readily upon the addition of thrombin, less so when mixed with tissue juice, and could not be regularly clotted by dilution, neutralization, or addition of calcium chloride. These differences vis-à-vis peptone plasma could be largely explained by the existence of an antithrombin in the peptone plasma on the one hand, and on the other by the presence of an antikinase in the venom plasma and perhaps also in the kinase plasma.

The different results obtained by Martin (327) stem from the probabality that not all snake venoms are alike in their physiological activity. It is quite possible that stronger or weaker hemolysis plays a part. Phisalix (336) hypothesized a relationship between the anticoagulant effect of snake venom, hemolysis, and the release of anticoagulant substances from the erythrocytes. This interpretation seems faulty since the effect is distinctly present in artificial coagulation systems in the absence of the formed elements, and also in rabbit blood in which there occurs but very little hemolysis. Furthermore, it has been general experience that the destruction of the red cells primarily produces substances which promote clotting. The action of Schmidt's "cytoglobin," as I have already theorized, is probably similar to that of cobra venom. It probably also contains an antikinase.

Finally, there exists a group of anticoagulants which

change in the protoplasm of the leucocytes. He thought that the protoplasm of the white cells could no longer be broken down, whereas the blood plasma remained unaltered and still possessed the ability to extract thrombin from normal protoplasm. The plasma coagulated upon the addition of normal lymphocytes or tissue juice.

The effect of the venom of an Australian snake (pseudechis porphyraceus) was very thoroughly investigated by Martin (327). He found a very significant correlation between the intravascular effect of the venom and that of the tissue juice. Large amounts of venom produced widespread intravascular coagulation, whereas that portion of the blood which was not clotted became incoagulable. Small amounts produced only the negative phase. This incoagulable blood resembled peptone blood since it still clotted upon the addition of thrombin and of tissue juice, and upon dilution with water. *In vitro* the venom had a moderately retarding effect upon coagulation. It did not contain nucleo-albumin (thrombokinase). Martin believed that the positive phase was brought about by a destruction of the formed elements. Stephens and Myers (343) found that cobra venom prevented coagulation *in vitro*, and that this could be nullified by the immune (antisnake) serum of Calmette.

According to my observations venom contains an antikinase. The effect of active thrombin present in serum was not materially diminished by cobra venom, but the coagulation of blood mixed with cobra venom at the time of collection was completely inhibited. There was a quantitative relationship between cobra venom and thrombokinase activity. The addition of sufficient thrombokinase overcame the anticoagulant effect. It is impossible to state whether or not the release of the thrombokinase from the formed elements was inhibited by the venom. Injections

the thrombins. However, the experimental conditions selected for the solution of this problem were not very convincing. This inhibitor could have been an antikinase. Bordet and Gengou (302) did not present any evidence for the presence of antithrombins in normal circulating blood.

The author (207) has made a few observations which seem to support the existence of such substances. Oxalate and fluoride plasma coagulated very poorly upon the addition of serum, a fact which had been observed by earlier investigators. This had been explained by the anticoagulant effect of oxalate. However, I have shown that this observation does not remotely suffice to explain the hypocoagulability of these plasmas. It must be assumed that there are anticoagulants present which inhibit thrombin almost quantitatively. The incomplete coagulation of oxalate and fluoride plasmas on the addition of a little thrombin is similar to the behavior of peptone plasma. This observation supports the hypothesis that such antithrombins exist. A natural assumption is that such antithrombins are not only present in extravascular plasma but also in the circulating blood.

The studies of Fuld (194), Loeb (436), and Muraschew (209) provided additional proof for the existence of an antithrombin in goose plasma, so that there exists little doubt that normal plasma contains anticoagulants as much as does goose plasma prepared without the addition of anticoagulants. Those substances apparently do not originate from vascular endothelium. Loeb was unable to extract anticoagulants from vascular endothelium and found only thrombokinase. Nothing conclusive can be said about the properties of these anticoagulants since knowledge concerning them is fragmentary.

Should antithrombins actually exist in the circulating blood, as appears highly probable, much would be gained

probably possess greater physiological significance than all the preceding ones and of which unfortunately very little is known. These are the antithrombins of tissues and blood.

Alexander Schmidt (54) postulated that the presence of clot-retarding substances was probably necessary for the preservation of the fluid state of the blood, but he was unable to demonstrate "cytoglobin" in the circulating blood. His disciple, v.z. Muehlen (80), discovered that so-called "kinase plasma" coagulated on prolonged dialysis and on the addition of sodium chloride. Schmidt found that it was possible by dialysis to remove substances from the blood which prevented the activation of metathrombin by alkali. His data were confirmed by Fuld (194) and myself (208). According to Schmidt anticoagulants were very probably present in the circulating blood.

Conradi described an anticoagulant in autolyzed tissue juice. During antiseptic autolysis the thrombokinase activity of tissue juice was rapidly reduced whereupon the latter soon displayed distinct anticoagulant properties *in vitro.* Autolysed tissue juice from lymph glands, ovaries and yeast cells were most effective in this respect. The inhibitor was heat-resistant, dialyzable, and could be precipitated by alcohol. Conradi was unable to demonstrate the presence of this inhibitor in the blood, nor did he find a heat-resistant antithrombin forming during the autolysis of blood.

By means of immunizing injections of rabbit serum into guinea pigs Bordet and Gengou (302) produced a relatively heat-resistant but weak inhibitor which reduced or inhibited the coagulant effect of rabbit serum on goose plasma. This inhibitor was effective only against the clot-accelerating substances present in rabbit serum. This finding furnished a clue concerning the species specificity of

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Specificity	*In Vitro and In Vivo Activity. Immunology*	*Substances Which Can Clot the Incoagulable Plasma (Containing the Anticoagulant)*	*Incoagulable Plasma Contains: 1. Prothrombin 2. Thrombokinase*
Apparently acts non-specifically against the thrombin of all animals	Equally active *in vivo* and *in vitro*. Immunity can be demonstrated *in vivo*	Thrombin (consistently), kinase (occasionally), (weak extract-plasma). No nucleoprotein ppt. on cooling	Prothrombin + Thrombokinase (?)
Specific data lacking. Most likely has no clear-cut specificity	Brought about by a vital reaction of the body (liver) following an injection of peptone Striking immunity	Thrombin in sufficient amount Tissue juice, distilled water, neutralization, calcium bring about rapid clotting. Nucleoprotein ppt. on cooling	Prothrombin + Thrombokinase +
Unknown	Most likely present in circulating plasma. Presence *in vitro* has been proved	Present in oxalate, fluoride, and goose plasma.	——
Unknown	Anticoagulant activity *in vitro* *In vivo* brings about positive phase of coagulation	——	——
Unknown	Anticoagulant activity *in vitro* and *in vivo*	Cell nuclei, but not by thrombin, water, acetic acid, CO_2, or $CaCl_2$	?
Pronounced specificity	*In vitro* activity proved. Immunity produced by injection of foreign serum	Normal serum heated at 58°C brings about clotting in goose plasma. Immune serum not effective	——
Unknown	Very active *in vitro*. Only slight prolongation of coagulation *in vivo*	Thrombin and "zymoplastic agent"	——
Precise studies lacking. Most likely not marked	Equally active *in vitro* and *in vivo*	Thrombin, kinase, and at times with distilled water, $CaCl_2$, acetic acid	Prothrombin + Thrombokinase (probably)
Unknown. Kinases are relatively specific (Loeb, Muraschew)	Produced by an injection of tissue juice by means of a vital reaction on the part of the body	Rapid clotting by thrombin. Poor clotting by kinase. Mixture with peptone plasma produces clotting	Prothrombin + Thrombokinase (?)

TABLE II

Anticoagulants

Type of Anticoagulant	*Mode of Action*	*Conditions of Neutralization*	*Chemical Properties*
Leech extract inhibitor (= hirudin)	Antithrombin	Neutralizes thrombin quantitatively. No influence on kinase	Heat-stable, precipitated by alcohol, water soluble, non-dialyzable
Peptone plasma inhibitor (crab muscle extract and eel serum should work by same principle)	Antithrombin	Probably neutralizes thrombin quantitatively. No influence on kinase	Heat-labile in solution (?), heat-stable in dry form, non-dialyzable
Inhibitor in normal (circulating) blood*	Antithrombin	Neutralizes small amounts of thrombin	Probably precipitated with the globulins. Dialyzable (?)
Inhibitors produced by autolysis (Conradi)	Antithrombin (probably)	Unknown	Heat-stable, precipitated by alcohol, dialyzable
Histone hydrochloride from the thymus gland (Lilienfeld)	?	Should be neutralized by (cell) nuclei	——
Inhibitor of Bordet and Gengou	Probably antikinase	Probably neutralizes thrombokinase quantitatively	Heat-labile Non-dialyzable
Cytoglobin of Alexander Schmidt	Antikinase	Probably neutralizes thrombokinase quantitatively but not thrombin	Dialyzable. Water soluble. Coagulated by alcohol
Cobra venom inhibitor	Antikinase	Neutralizes kinase, but not thrombin. In turn is neutralized by immune snake serum	Unknown
Inhibitor produced by injection of kinase (tissue juice)	Probably antikinase	Neutralizes kinase but not thrombin	Unknown

* This inhibitor has been detected in oxalate, fluoride, and goose plasma

Chapter VII

CLOT-PROMOTING SUBSTANCES (CALCIUM AND GELATIN)

While the number of substances which inhibit or delay coagulation is very great, it is unfortunate that only a few substances are known which promote coagulation without simultaneously producing thromboses in the circulating blood. Even now the physician possesses few means to increase the coagulability of blood which would be highly desirable in diseases like hemophilia, hemorrhagic diatheses, leukemia, and aneurysms. The intravenous injection of thrombokinase to increase the coagulability of the blood is naturally entirely out of the question since it would result in intravascular thromboses, or might even result in a total reversal of the desired effect by rendering the blood incoagulable. Local application of thrombokinase is theoretically justified as a means of stopping bleeding. However, it has not yet been possible to produce very potent, aseptic and stable solutions. The local application of thrombin is not very promising because of the impotency of the available solutions.

Experience with the internal use of calcium salts as a means of increasing coagulability has been favorable. Wright (177) was the first to recommend the use of calcium chloride. Boggs claimed that the internal use of calcium chloride, or even better calcium lactate, shortened the coagulation time whether these compounds were taken orally or intravenously (351). However, the results were not very striking, particularly when the oral route was used. It is

toward an understanding of the liquid state of the blood. It must be admitted, however, that the maintenance of the liquid state of the blood can also be explained in the absence of antithrombins. According to the studies of Bordet and Gengou (185), the plasmatic precursor of thrombin yields thrombin rapidly only in the presence of wettable foreign surfaces. A reasonable assumption is that despite the presence of prothrombin and small amounts of thrombokinase in blood plasma, no thrombin is formed in the circulation.

Loeb's discovery that incoagulable phosphorus blood did not contain an inhibitor seemed to contradict the thesis that antithrombin is present in circulating blood. Phosphorus blood, however, seems to lack other enzyme inhibitors. Its chemical composition differs so markedly from the normal that studies of this plasma might best be omitted.

The investigations of the anticoagulants discussed in the preceding chapter show clearly that we are still in the dark in this field. It is very probable that anticoagulants play a much more significant role in normal coagulation than was assumed in the past. Many observations which have been difficult to interpret can probably be best explained by the presence of such substances. One example is the marked variations in the coagulation time determination. Table II giving the type and mechanism of action of various anticoagulants has been taken from a paper by the author (329).

injections of small amounts of gelatin (0.4 gm per kg). Lancereaux and Paulesco (374) demonstrated later that the clot-promoting effect was present not only after intravenous but also after subcutaneous injections of gelatin.

Naturally such an important discovery was immediately seized upon with great interest. There have been many clinical reports concerning the use of subcutaneous gelatin for hemorrhage, most of them favorable. It is therefore all the more striking that experimental attempts to verify the results of Dastre and Floresco led to opposite conclusions. Camus and Gley (355) attributed the results of Dastre and Floresco to the acid content of the gelatin. They denied the favorable results of subcutaneous or intraperitoneal gelatin injections. In general negative findings were also obtained by Tóvoelgyi (390), Mariani (377), Sackur,* and Steensma (389). Indeed, Brat (353) found that glutone inhibited or delayed coagulation just as peptone did. Zibell (397) and Gley and Richaud (368) attributed the slight acceleration of coagulation to the calcium content of gelatin which amounted to 1 per cent or greater. In checking the findings of Dastre and Floresco, Boggs (351) was unable to obtain consistent results. In a number of experiments with subcutaneous and intravenous injections into rabbits Boggs noted a distinct shortening of the coagulation time from 6 to ½-1 minutes. These findings were well outside of the margin of error. This reduction in clotting time lasted several days. On the other hand, similar experiments yielded negative results.

The attempts of other investigators to confirm and clarify the action of gelatin also yielded inconsistent results. Therefore, Gebele (366) concluded on the basis of clinical and experimental studies that parenteral gelatin

* Sackur: Gelatine und Blutgerinnung. *Mitteil. a. d. Grenzgeb. d. Med. u Chir*, *8*.188, 1901.

difficult to give a theoretical explanation for the effect of the calcium salts. The effect might be partly explained by the possibility that an increase in calcium ions in the blood accelerates the production and activity of thrombin. If such were the case, it would have to be assumed that blood does not normally contain the optimum amount of calcium salts necessary for coagulation.

Gelatin injections to improve coagulation have found much wider acceptance in medical circles. The literature in this field, especially the clinical reports, assumes that the experimental basis has been completely clarified and that all the clinical results are uniform. In truth there is no problem in the entire field of coagulation evoking a wider divergence of opinion than that concerning the effect of gelatin. No one knows how gelatin accelerates coagulation. Indeed, many investigators are not even sure that it does shorten the clotting time.

According to von Boltenstern (352) gelatin was widely used in former days as a local hemostyptic. It subsequently fell into disuse until Dastre and Floresco (360) in 1895 and 1896 discovered the clot-promoting propensities of an intravenous injection of gelatin. At the same time Carnot (356) also recommended gelatin as a local hemostyptic. During experiments concerning alterations in gelatin in the circulation, Dastre and Floresco noted a strong clot-accelerating effect following the injection of gelatin. Dogs weighing 15 kg were given intravenous infusions of 80 to 400 cc of an 8 per cent gelatin solution. Blood apparently coagulated at times within 10 seconds even though it was kept at 38°C, so that a simple congealing of the gelatin by cold was eliminated as the explanation for the phenomenon. They also found that gelatin had a clot-promoting effect *in vitro* although this was not as striking. The anticoagulant effect of peptone could be blocked by subsequent

Personally, I consider Kaposi's criticism of the earlier experiments more important than his later results. The contradictory findings of previous investigators can be partly explained by differences in the types of gelatin used. Brat's glutone, for example, is a gelatose. It has been known since the investigations of Arthus and Huber (218) that gelatoses behave like albumoses, i.e., as anticoagulants. Merck's liquid sterilized gelatin is also rich in gelatose and poorly suited for such experiments. The gelatins which are liquid at ordinary temperatures are recommended for local use only since the local effect is probably primarily based on the conglutination of blood cells. Only those gelatins which remain solid at ordinary temperatures should be given subcutaneously. This is a matter which must be watched with greater care in future experiments concerning the gelatin effect.

Another source of error is found in the clotting time determinations. Depending on the methods used the results of various authors differ considerably, even in regard to what constitutes the range of the clotting time of normal blood. This naturally creates certain difficulties in evaluating the gelatin effect.

Kaposi's criticisms, unfortunately, settled only part of the argument. Boggs experimented with a great variety of gelatins without success. The mistakes in clotting time determinations which were undoubtedly present also did not explain entirely the contradictory results of various authors. Boggs (351), who used the apparatus of Brodie-Russell (404), and Buerker (460) claimed that it was possible to obtain consistent results for the clotting time if a well-standardized method was used and differences in temperature and other factors were carefully controlled. This should preclude serious error, and only great differences in clotting time would be of importance in this question anyway.

did not promote clotting until the body had lost a large amount of blood, approximately one-fourth to one-fifth of the total blood volume. Under such circumstances there was an unexplained increase in the coagulability of the blood as the experiments of Arloing (398) and others had already demonstrated. Gelatin injections could then further shorten the coagulation time but were ineffective without prior extensive loss of blood. Moll, on the other hand, concluded that the clot-promoting effect of gelatin was not manifested so much by a reduction in the clotting time but rather that gelatin injections brought about leucocytosis and an increase of fibrinogen which was ascertained by the method of Reye (150). In Moll's opinion the *hemostatic effect consisted primarily in an increase in precipitated* fibrin and greater stability of the thrombus, whereas this was not necessarily accompanied by a reduction in the clotting time (381).

Shortly thereafter Kaposi (373) critically reviewed the studies on gelatin. His personal experience supported the view that gelatin was clot-promoting. He found that gelatin injections restored the coagulability of animal blood which had previously been rendered incoagulable by hirudin. He felt that the agglutination of blood corpuscles (already mentioned by Sackur*) which occurred upon the *addition of gelatin and other colloids might* very well staunch the flow of blood by plugging the lumina of smaller vessels. Furthermore, abolition of the hirudin effect by means of gelatin had already been unsuccessfully attempted by Steensma (389) and Boggs (351). Kaposi, in the belief that his experiments supported his theory of the clot-promoting effect of gelatin, maintained that gelatin was effective by increasing or accelerating the production of thrombin.

* See footnote page 131.

Chapter VIII

CHANGES IN THE MORPHOLOGICAL ELEMENTS OF BLOOD DURING COAGULATION

IT IS NOT my intention to deal with the extensive literature concerning morphological alterations occurring during coagulation. However, this report would be incomplete if it did not emphasize a few points which incidentally also play a significant role in the chemistry of coagulation.

There can no longer be any doubt that the cellular elements in one way or another participate in coagulation. Wooldridge's belief that all factors necessary for coagulation were present in the circulating plasma has never been widely accepted and can be dismissed with the explanation that Wooldridge worked with peptone plasma which differs markedly from normal circulating plasma. All other observations support the idea that the formed elements participate in coagulation. One need only think of the situation found in goose plasma, the coagulation of sedimented horse plasma in which the first clots are formed within and above the layer of the formed elements, and other phenomena, which could hardly be explained unless one assumed the participation of the formed elements.

Certain questions remain: What do the formed elements release into the plasma? Which of the formed elements are exclusively or primarily involved? Both questions have been answered partially by chemical and morphological studies so that some conclusions can be given.

The foregoing shows that the ideas concerning gelatin, more than any other aspect of blood coagulation, need precise experimental study. It should first be ascertained whether gelatin *in vitro* or *in vivo* actually accelerates clotting in normal individuals or under specific experimental conditions. Only when this question has been satisfactorily answered, can an attempt be made to investigate the cause of the phenomenon.

Finally, it should be mentioned that Landau* isolated a clot-promoting substance, "stagnin." Since this was obtained by autolysis of splenic tissue, it should contain the antithrombin of Conradi (358) which is said to form during autolysis. Further information concerning the effect of stagnin is lacking.

* Landau, T.: Ein neues, durch Autolyse der Milz gewonnenes Blutstillungsmittel (Stagnin). *Berliner klin. Wchnschr., 41*:577, 1904.

takes place prior to or simultaneous with coagulation. This question remains undecided. The studies of Dastre (461, 462), Arthus (181), Ruechel and Spitta (483), and Bayon (458) (despite the energetic opposition of Krueger [79]) were responsible for the belief that no breakdown of leucocytes occurred in normal coagulation and that the minution of leucocytes in peptone blood did not necessarily imply destruction of these elements. It has always appeared to me that as Schmidt has occasionally intimated the entire problem of the disintegration of the leucocytes is not as important as many have thought. The hypothesis that the formation of thrombin is ultimately a function of *the leucocytes is not impaired by the absence of breakdown* of these elements. The possibility remains that there is secretion of the active agent without gross anatomical change.

Are we at all certain that the leucocytes actually play the important role in coagulation which many investigators have ascribed to them? Prior to the discovery of the blood platelets the participation of the leucocytes in coagulation could be reasonably accepted since certain phenomena such as the beginning of coagulation in the leucocytic layer of sedimented, cooled horse blood were otherwise inexplicable. Even after the discovery of the platelets the possible participation of the leucocytes in coagulation could not be denied. Goose plasma in which no platelets have been found, often coagulated spontaneously without the addition of tissue juice. In this case after prior sedimentation, coagulation began in the leucocytic layer, which occasionally could be lifted out as a flat saucer-shaped clot, while the plasma above it and the layer of blood cells underneath were still completely liquid. Even more impressive were observations on the coagulation of lymph which often did not contain formed elements other than

It has already been pointed out above that of the substances needed for coagulation, fibrinogen and calcium salts are present in the circulating plasma whereas thrombin is lacking. If the classical theory of the thrombin nature of coagulation (which best fits known facts) is to be taken as a point of departure, the significance of the formed elements must be sought chiefly in the release of one or both of the precursors of thrombin. The origin of thrombokinase from the formed elements can hardly be doubted in view of the existence of types of plasma such as goose and fluoride plasma which do not contain sufficient thrombokinase and therefore remain liquid. The question concerning the pre-existence of prothrombin in circulating blood is much more difficult to answer. However, as mentioned previously, there exists no compelling reason to doubt the pre-existence of this substance since all plasmas, regardless of their origin, contain prothrombin. My suggestion (480) that prothrombin originates from platelets still needs verification for reasons previously discussed. Tentatively the assumption can be made that prothrombin exists in circulating plasma whether it originates from the formed elements of the blood or from certain organs. The function of the formed elements, therefore, is that of releasing thrombokinase into the plasma.

In order to decide which of the formed elements is chiefly or exclusively concerned with coagulation one must return to the older studies of Alexander Schmidt and Mantegazza (140). Both men were of the opinion that the leucocytes were the chief agent in coagulation. Schmidt stated that thrombin or the "zymoplastic agent" originated from leucocytes and were released by the extravascular breakdown of these cells.

Both assumptions have been attacked. Many authors remain unconvinced that disintegration of the leucocytes

lation was inhibited by appropriate measures, such as dilution with a calcium solution. This situation may, according to Loeb, explain the low fibrin content of platelet thrombi.

Even though the agglutination of platelets need not always go hand in hand with coagulation, this observation still indicates the probable participation of these elements. This is all the more likely since as shown earlier, platelets like other protoplasmic material, contain thrombokinase. The question arises as to which formed element is more important in normal coagulation, leucocytes or platelets. The problem of the origin of platelets will not be taken up here since it is of little significance for this discussion.

In my opinion most observations indicate a greater significance of platelets than leucocytes in the coagulation of mammalian blood. First of all it must be accepted that platelets disintegrate during coagulation and that unlike leucocytes they are extremely unstable elements. Is the disappearance of the platelets a cause or a result of coagulation? Offhand the first possibility does not appear likely since both prothrombin and thrombokinase are present in oxalate plasma even though platelets appear quite well preserved. There is no proof that the disintegration of platelets is absolutely necessary for the origin of thrombin.

Microscopic observation has shown that platelets frequently form so-called clotting centers, i.e., foci from which the formed fibrin threads emanate. This observation is not very informative, because Bordet and Gengou (185) found that any foreign body can form such a clotting center in cell-free plasma obtained by centrifuging blood in paraffined vessels.

Nor do the observations of Pratt (482) concerning the number of platelets and the clotting time demonstrate any relationship between the two. Nevertheless, one must note

white blood cells. Loewit (478), Mosen (481), and others were unable to demonstrate the presence of platelets in lymph. There can then be no doubt that coagulation can occur in fluids which contain only leucocytes and no other formed elements.

This is borne out by the interesting observations on coagulation in the lower animals for which we are indebted to men like Griesbach (466, 467), Bottazzi (403), Ducceschi (416), and in particular Loeb (434-437). In certain crustaceans (limulus) coagulation occurred in two stages. The first stage consisted of changes in the leucocyte-like amoebocytes of the blood which upon contact with foreign surfaces, extend pseudopods which in turn were woven into a dense mesh (plasmoschise). The second stage resembled more closely coagulation in vertebrates. A clot formed which seemed to depend on the precipitation of a fibrin-like substance from the plasma. This second stage of coagulation might be brought about by an enzyme. It is similar to coagulation in higher animals since it could be prevented by calcium-precipitating substances. On the other hand the first stage, the agglutination of the amoebocytes occurred in the absence of oxalate-precipitable calcium salts according to Loeb.

Observations made on invertebrates can be applied to vertebrates only with extreme caution. Some analogous conclusions, however, appear justified since the investigations of Ducceschi (416) and Loeb indicated that vertebrates do not completely lack the first phase of coagulation which occurs in the invertebrates. Shortly before coagulation there appeared in vertebrate blood macroscopically visible, miliary clumps which adhered to the vessels and consisted chiefly of platelets, and to a lesser extent of leucocytes. Coagulation did not necessarily follow this agglutination. The agglutination occurred even when coagu-

regard to the significance of the platelets (454). This granular substance is undoubtedly of great importance in coagulation.

As yet there is no absolute proof that in normal coagulation of mammalian blood the platelets rather than the leucocytes are of chief importance in the production of thrombin. On the other hand it can be taken for granted that the leucocytes also participate in coagulation since fluids which contain only leucocytes also clot even though more slowly.

That the erythrocytes have a significant role has not been convincingly demonstrated. I was able to show that the stroma of the red cells contains thrombokinase (208). However, it is doubtful that the erythrocytes play an important role in normal coagulation. There is no need for this assumption, unless one believes, with Arnold and his followers (11) that the platelets originate primarily from the erythrocytes.

This short synopsis shows that the modern coagulation theory not only harmonizes numerous contradictions regarding the chemical aspects of coagulation, but that it is also able to eliminate much of the sharp divergence of opinion resulting from morphological investigations. No one formed element is of exclusive importance in coagulation. It is no longer a question of leucocytes *vs. platelets since both as producers of thrombin can take* part in coagulation.

that prolonged clotting and platelet deficiency occur together in some pathological situations, an observation the author himself was once able to verify.

Other observations support the importance of the platelets. One of these concerns the previously mentioned fact that only those body fluids which contain platelets coagulate quickly without the addition of tissue juice whereas goose plasma and lymph are distinguished by slow coagulation. The slow clotting of lymph must not be ascribed to its low fibrinogen content since other fluids with low fibrinogen concentration coagulate rapidly upon addition of adequate thrombin according to Dastre (96).

The significance of the platelets has also been demonstrated by the experiment of Bizzozero, who was the first to attempt to render blood platelet-free. This was done by numerous phlebotomies and re-injection of the blood after it had been whipped. The blood which now circulated contained virtually no platelets and coagulated only very slowly which nevertheless indicated that it still contained some fibrinogen. The author can vouch for the slow coagulation of such blood. It is, however, uncertain that prolonged coagulation can be primarily attributed to the reduced number of platelets since the experimental conditions were very complicated.

More convincing is the experiment of Buerker (460). He caught a drop of blood on a paraffin block. No coagulation occurred. On gradual sedimentation the lighter platelets collected in the upper part of the drop. When the tip of the drop was touched with a cover-glass, microscopic observation showed that the formed elements in the plasma were almost exclusively platelets. Coagulation then followed very rapidly. Wooldridge's beliefs concerning "fibrinogen-A" which was precipitated from the plasma as platelet-like granules should be further mentioned with

regard to the significance of the platelets (454). This granular substance is undoubtedly of great importance in coagulation.

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Chapter IX

CONCLUSION

In conclusion the present concept of blood coagulation can be summarized as follows: Fibrinogen, calcium salts, and probably also prothrombin as well as a limited amount of anticoagulants (antithrombins) are present in the plasma of the circulating blood. The formed elements, platelets and leucocytes, contain thrombokinase. The constant disintegration of formed elements in the circulating blood probably releases a small amount of thrombokinase into the plasma. This amount is either so small that the available antithrombins suffice to inactivate the resulting thrombin, or thrombokinase can not combine with the calcium salts and prothrombin to form thrombin in the absence of a wettable foreign surface. Both possibilities are acceptable. In the event of damage to the vascular wall which renders it a wettable foreign surface there occurs in this locale an agglutination of platelets and leucocytes which need not necessarily lead to true coagulation if the lesion is minor.

The manner and extent to which coagulability of the blood is influenced by the organs through which it flows can not presently be stated. That the parenchymatous organs contribute to the maintenance of the liquid state of blood and possibly also to its coagulability is highly probable in view of observations of the peptozyme effect and other phenomena.

When the blood leaves the vessels it comes in most

instances in contact with wettable foreign matter like tissues or other surfaces. Once again agglutination of the platelets and of a number of leucocytes occurs. But the stimulus of contact with the foreign surface continues and results in a massive release of thrombokinase into the plasma. When blood establishes contact with destroyed tissue, such as the surface of a wound, thrombokinase need not exclusively come from the formed elements of the blood. It can partly emanate from the destroyed cells themselves in which case coagulation occurs all the more rapidly. This phenomenon apparently plays an important role in the spontaneous coagulation of avian blood following injury.

When thrombokinase is released into the plasma, it activates prothrombin in the presence of calcium salts. This process is augmented by the presence of foreign bodies, mechanical influences, etc. Thrombin is formed in such amounts that the available anticoagulants no longer suffice to inactivate it. In normal coagulation only part of the prothrombin is converted to thrombin. The formed thrombin begins to alter the fibrinogen in which case there probably first forms an intermediate liquid product and subsequently solid fibrin. As the production of thrombin continues, the first signs of coagulation appear. The production of thrombin is then suddenly halted, either because the supply of thrombokinase is exhausted, or because inhibitors again become operative or augmented. The large amount of thrombin produced during coagulation then disappears except for a slight residuum whereupon very little thrombin is found in the serum. That part of the thrombin which adheres very solidly to the resulting clot is removed with the latter. The greatest part, however, is converted rapidly to an inactive form of thrombin, metathrombin. The blood serum squeezed out

of the clot therefore contains a small amount of thrombin and large quantities of prothrombin and metathrombin. In addition anticoagulants probably exist in the serum as well as thrombokinase, and these substances may balance each other.

The ideas discussed here are not necessarily definitive. They best express the known facts interpretated on the basis of the prevalent belief in the enzymatic nature of coagulation.

A SELECTED GLOSSARY

Note: *A standard index for this monograph seemed of little value since the important terms were used repeatedly throughout the text. A glossary appeared to be of more benefit, chiefly to explain that part of the terminology now obsolete. Several of the more important topics not noted in the table of contents are indexed in the glossary. A few general medical terms are defined to aid the biologist and the biochemist. With respect to certain terms used repeatedly throughout the text, no attempt was made to list all of the numerous pages on which they occurred*

A

Agglutination (p. 132): The collection into clumps of cells distributed in a fluid.

α-prothrombin (p. 64): Obsolete term. Morawitz originally used this term to refer to plasma prothrombin in contrast to metathrombin present in serum after coagulation See β prothrombin.

Albumin (p. 9)· A modern definition would be that plasma protein soluble in one-half saturated solution of ammonium sulfate in contradistinction to globulins which are insoluble under such circumstances. Albumin constitutes approximately 60% of proteins in normal human plasma. A former loose definition embraced the generally more soluble portion of plasma proteins in the sense that they were less easily precipitated by a variety of salt solutions

Albumose (pp. 42, 99, 118): Any primary product of the digestion of an albumin. Differs from albumin in not being coagulable by heat. The albumoses are converted by further digestion into peptones.

Alexines (p. 120)· Non-specific thermolabile ferment-like substances found in blood plasma. In the presence of a specific sensitizer they exert a lytic action on bacteria and other cells. Also called complement. Leucocytic alexin (leukin) by contrast is relatively heat-stable. In general the use of these terms is now obsolete.

Amino acids (pp. 27, 117): Organic acids in which one or more hydrogen atoms have been replaced by an amino group. One variety, the α-amino acids, are considered

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for Schmidt's claim that it is a precursor of fibrinogen. Considered to be only contaminated nucleoprotein by Morawitz.

Cytozyme (p. 68): Fuld's term for the clot-promoting substance in tissues. Probably corresponds to thromboplastin or thrombokinase.

D

Ductus Choledochus (p. 106): Common bile duct.

E

Eck Fistula (p. 106): An artificial communication between the portal vein and the inferior vena cava.

Endothelium, Vascular (p. 125): *Refers to the layer of cells lin*ing the inner surface of blood vessels.

F

Fibrin (p. 4, Chapt. V, p. 49). A whitish, insoluble protein formed from fibrinogen by the action of thrombin (fibrin ferment) as in the clotting of blood. Fibrin forms the essential portion of the blood clot.

Fibrin Ferment (pp. 11, 15, Chapt. IV): Schmidt's original term for thrombin.

Fibrin Generators (pp. 12, 15): Refers to the two substances (fibrinogen and "fibrinoplastic substance") which according to Schmidt's first coagulation theory combined chemically to form the fibrin clot. An obsolete theory.

Fibrinocrasias: See p. 77.

Fibrinogen (pp. 7, 11, Chapt. V., p. 79): A soluble protein in blood plasma which is converted into the fibrin clot by the action of thrombin (fibrin ferment).

Fibrinogen-A (pp. 61-63, 101-102, 140): Wooldridge's term for a substance obtained as a granular precipitate from peptone plasma after prolonged cooling at 0°C. This was probably really an accumulation of blood platelets.

Fibrinogen-B (pp. 61, 102): Wooldridge's term which corresponds to the plasma fibrinogen of other workers.

Fibrinoglobulin (pp. 23, 90, 91): Term used by Hammarsten for a globulin-like product of fibrinogen found in blood serum after coagulation. Probably either corresponded to "soluble fibrin" or was a nonspecific contaminant.

Fibrinoplastic Substance (pp. 12, 19, 29): Term originally used by Schmidt to denote the substance in blood serum which produced clotting by combining with fibrinogen. Schmidt thought this substance originated from the formed elements of blood. The fibrinoplastic substance was obtained from diluted blood serum by precipitation with CO_2. Actually this was probably serum globulin (older term "paraglobulin") and any coagulant activity measured in Schmidt's experiments was likely due to contaminating thrombin. Schmidt dropped the theory of the fibrinoplastic substance after his discovery of fibrin ferment (thrombin).

to be the basic "building blocks" of proteins.

Amoebocytes (p. 138): Large nucleated blood cells in certain lower animals which may have functions similar to the mammalian platelet in sealing rents in vessels by agglutination.

Anchyclostomum Caninum (p. 121): Hookworm, with reference to the type infesting dogs.

Aneurysm (p. 129): A sac formed by dilatation of the walls of an artery. Usually filled with blood or blood clots.

Antibody (p. 111): A specific substance produced in and by the body as a reaction to the presence of an antigen.

Antithrombin (pp. 103, 109, 111): A substance which inhibits the action of thrombin.

Apneic (p. 115): Refers to the transient cessation of breathing

Ascites (p. 72): The accumulation of serous fluid in the abdominal cavity.

Autolysis (pp 85, 124, 126): The spontaneous disintegration of tissues or cells by the action of their own (autogenous) enzymes.

B

β-prothrombin (p. 65): Morawitz' original term for metathrombin.

C

Casein (pp. 20, 39, 99): A phosphoprotein, the principal protein of milk, and the basis of curd and of cheese. Casein is changed by rennin into paracasein which in the presence of calcium forms an insoluble curd.

Caseoses (p. 99): A proteose produced during the digestion of casein. Obsolete term.

Cell Fibrinogen (pp. 53, 63): See tissue fibrinogen.

Cell-globulin (p. 53): An obsolete term for the clot-promoting substances in tissues. In a generic sense probably corresponds to tissue thromboplastin.

Chickenfat Clot: See p. 92.

Chyle (p. 17): The milky fluid taken up by the intestinal lacteals after digestion. It consists of lymph and emulsified fat. It passes into the blood stream via the thoracic duct.

Coeliac Plexus (p. 106): Same as the solar plexus. A network of nerves and ganglia adjacent to the stomach and giving off nerves to all of the abdominal organs.

Conglutination (p. 133): Agglutination which is augmented by colloids such as plasma proteins, gelatin, etc.

Crotalus (p. 121): A genus of rattlesnakes.

Crustaceans (p. 138): A large class of animals including certain shell-fish and water fleas.

Crusta Inflammatoria: See p. 92.

Cytoglobin (pp. 31-34, 43): Term used by Alexander Schmidt for an anticoagulant substance isolated by aqueous extraction of alcohol-dried cells. Considered to be albumin-like and to contain a large amount of phosphorus Probably has no role in physiological coagulation. No evidence

in the tunica vaginalis of the testicle.

I

Ichthyotoxicum (p. 112): A poisonous principle which can be obtained from various fish.

K

Kinase Plasma (pp. 115, 116, 121, 126): The plasma obtained following the intravenous injection of thrombokinase (thromboplastin).

Koagulin (pp 53, 60, 75): Clot-promoting substance in tissue isolated by Loeb, who considered its activity to be thrombic in nature. Probably corresponds to thrombokinase.

L

Laked Blood (p. 24): A term applied to blood in which the hemoglobin has been separated from the red cells.

Lecithin (p. 62): A phospholipid. Present particularly in nerve tissue, semen and to a lesser extent in blood and bile.

Leucolysis (p. 108): The destruction of leucocytes.

Leuconuclein (pp. 42, 108): Although this substance was originally considered to be an acid nucleoprotein, it was probably simply a nucleic acid. The apparent protein nature may have been merely due to failure to remove contaminating protein. Lilienfeld ascribed a procoagulant activity to leuconuclein. At present this would probably be described generically as tissue thromboplastin. Obsolete term.

Leucopenia (p. 27): Reduction in the number of white blood cells.

Limulus (p. 133): Pertains to king crabs.

Lymph (pp. 13, 17, 107): A transparent, slightly yellow liquid which fills the lymphatic vessels. It is occasionally light-rose in color due to red cells or opalescent due to fat particles.

M

Mast Cells (p. 112): Tissue basophils. Large cells containing coarse basophilic granules. They occur particularly in connective tissue. These cells are thought to contain heparin, the anticoagulant present in peptone shock.

Metathrombin (pp. 65-67, 71, 143): A hypothetical substance into which thrombin changes on standing following coagulation. It may again be activated to thrombin by the action of alkalis or acids.

Metazyme (p. 65): Probably corresponds to metathrombin

Miliary (p. 138): Resembling a millet seed. Refers to small, scattered particles, substances or lesions.

Myosinogen (p. 54): Obsolete term for a protein now called myosin which is present in muscle.

N

Naja (p. 121): Cobra di capello (hooded cobra)

Negative Phase (pp. 61, 115, 122, 123): Impaired coagulability of the blood following intravenous injection of certain substances, e.g., tissue extracts.

Fibrinolysis (pp. 84, 93-95): The splitting up of fibrin by enzymatic action.

Fibrinolytic Enzyme (p. 10): Any enzyme which produces fibrinolysis.

Formed Elements (p. 16): The solid components of blood (red and white cells, platelets) as contrasted to the liquid plasma.

G

Gelatose (pp. 99, 133): An albumose formed by hydrolyzing gelatin by acids, alkalis, or enzymes.

Globulin: A class of proteins characterized by insolubility in water and solubility in some weak neutral salt solutions. (In modern terminology this would apply only to euglobulins. Another variety, pseudoglobulins, is soluble in water but not in one-half saturated ammonium sulfate solution.) Serum globulins constitute about 40% of the plasma proteins in health. Serum globulins were formerly referred to as "paraglobulin."

Globulin A (p. 54): Substance isolated by Halliburton from lymphocytes. This substance had no thrombic activity in contrast to Globulin B.

Globulin B (p. 54): Substance isolated by Halliburton from lymphocytes which he claimed was identical with thrombin. This substance, however, probably corresponded to thromboplastin (thrombokinase).

Glutone (pp. 131, 133)· A gelatose.

Gypsum (p. 35): Hydrous calcium sulfate. The mineral from which plaster of paris is made.

H

Heat-coagulation: See footnote, p. 19.

Hemophilia (pp. 76, 129): An hereditary disorder characterized by delayed clotting of the blood and consequent difficulty in checking hemorrhage. Typically it is inherited by males through the mother as a sex-linked character.

Hemostatic (p. 132): Refers to the arrest of (excessive) bleeding.

Hemostyptic (p. 130): Same as hemostatic.

Heparin (p. 111): An acid mucopolysaccharide occurring in various tissues, but most abundantly in liver and lung. Acts as a potent anticoagulant.

Hetero-albumose (p. 100): A mixture of albumoses. Obsolete term.

Hirudin (p. 117): The anticoagulant of the leech.

Histone (pp. 42-44, 108, 109, 121): A simple protein soluble in water and insoluble in dilute ammonia Combined with leukonuclein it forms nucleohistone. Blood treated with large amounts of histone is altered so that it coagulates with difficulty. See also nucleohistone and leukonuclein.

Humoral Pathology (p. 3): Refers to the obsolete doctrine that all disease arises from some imbalance in the body humors (blood, phlegm, yellow bile, and black bile).

Hyaline (p. 81): A glassy, translucent (or almost translucent) substance.

Hydrocoele (pp. 8, 12, 35, 72): A circumscribed collection of fluid

in the tunica vaginalis of the testicle.

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Ichthyotoxicum (p. 112): A poisonous principle which can be obtained from various fish.

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Nucleo-albumin (pp. 53-55): Older term used for nucleoprotein.

Nucleohistone (pp. 42, 43, 53): See histone and leuconuclein. Nucleohistone was thought to be the combination of these two substances. Obsolete term.

O

Optical Rotation (p. 80): The rotation of the plane of polarized light by the asymmetry of a molecule. This property is specific for each substance and can be used for its identification.

P

Paracasein (p. 39): Casein is converted into paracasein by the action of rennin. In the presence of calcium, paracasein is then precipitated as an insoluble curd.

Paraglobulin (pp. 10, 19, 29, 32): Commonly refers to the serum globulins. Also at times used in reference to globulins in blood cells, lymph, and connective tissue. Obsolete term.

Parenchymatous (p. 116): Pertaining to parenchyma, the essential or functional elements of an organ as distinguished from its stroma or framework.

Parenteral (p. 131): Not through the alimentary canal, i.e., either subcutaneous, intramuscular, or intravenous.

Peptone (p. 62, Chapt. VI, pp. 96-112): A product of extensive hydrolysis of a native protein by an acid or by an enzyme.

Peptone Plasma: The hypo- or incoagulable plasma obtained following an intravenous injection of peptone.

Peptone, Witte's (p. 98): A proprietory dry peptone prepared from fibrin.

Peptozyme (pp. 100, 104, 105): The hypothetical active principle in peptone thought to produce the coagulation defect (heparin) in peptone shock.

Pericardial (p. 12): Pertaining to the pericardium, the membranous sac which invests the heart.

Peritoneal (p. 12): Pertaining to the serous membrane which lines the abdominal wall and invests the contained viscera.

Plasmin: See p. 10.

Plasmoschise: See p. 138.

Plasmozyme (p. 68): Older term for prothrombin used by Fuld.

Platelet (p. 137): A blood platelet; one of the small colorless corpuscles in the blood of all mammals in the form of oval or round discs approximately 3 μ in diameter. Presumably derived as fragments of cytoplasm from the megakaryocytes of bone marrow.

Portal System (p. 114): Pertains to the venous drainage system of the abdominal organs.

Positive Phase (p. 62): Pertains to the production of intravascular thromboses following the intravenous injection of certain substances (e.g., tissue extracts) in contrast to the impaired coagulability (negative phase) seen at other times.

Preglobulin *(p. 32): A protein* Schmidt derived from cytoglobin by decomposition with acids. Schmidt considered preglobulin a

precursor of fibrinogen, but this was an erroneous theory.

Pro-enzyme (p. 65): The precursor of an enzyme.

Propeptone (p. 100): Term used by Grosjean for a product of the digestion of certain proteins.

Proplastic Fluids: See pp. 12, 13, 29, 45.

Prothrombin (p. 30): The popular, modern term for the plasmatic precursor of thrombin. (Also called thrombogen, serozyme).

Protozyme (p. 26): Rauschenbach's term for a precursor of fibrin ferment (thrombin) which he postulated existed in all tissues. Probably corresponds to thrombokinase.

Pseudechis porphyraceus (p. 122): Australian black snake.

Pseudopod (p. 138): A temporarily protruded portion of the cytoplasm of a cell.

R

Rennin (p. 11): The milk curdling ferment found chiefly in gastric juice Also called chymosin.

Retraction, Clot: See p 92.

S

Serofibrin (p 10)· Term Denis originally used for what he subsequently called "plasmin" and eventually correctly termed fibrinogen.

Serous Fluids: See p. 12.

Serum Fibrinogen (p. 62): Name given by Wooldridge to a substance isolated from blood serum by acid precipitation. Exact nature of this substance is not clear.

Serum Globulin (p. 14): See globulin.

Soluble Fibrin (pp. 8, 17, 22): As initially used by Buchanan this probably implied unaltered fibrinogen. As used by subsequent workers it designates an intermediate product in the conversion of (liquid) fibrinogen to the solid clot (fibrin). Also called profibrin

Stagnin: See p. 134.

Stroma (p 28): The tissue which forms the ground substance, framework or matrix of an organ; also that portion of the red blood cell which remains after hemoglobin and fluid have been removed.

Stromafibrin (p. 81): A term that has been applied to a fibrinogen-like substance extracted from red blood cells with dilute salt solution.

T

Thrombases (p 96): Originally used to denote artificial anticoagulants by Duclaux. Also has been used synonymously with thrombin.

Thrombin (pp. 11, 30, Chapt. IV, p 51) The enzyme present in shed but (presumably) not in circulating blood which converts fibrinogen to fibrin Synonym· fibrin ferment

Thrombogen (pp. 68, 72): Synonym for prothrombin.

Thrombokinase (pp. 30, 68, 114): The substance which acts to convert prothrombin to thrombin. Synonymous with thromboplastin, but certain workers prefer to use the suffix "-ase" to emphasize their belief in the

enzymatic nature of the process. Synonyms: "zymoplastic substance," "cytozyme."

Thromboplastin (p. 30): In general now used synonymously with thrombokinase.

Thrombosin (pp. 42, 46, 49): A principle derived from fibrinogen and itself convertible into fibrin. Lilienfeld and Arthus believed that fibrin was a calcium salt of fibrinogen or a "thrombosin." Hammarsten later showed that thrombosin was nothing other than fibrinogen precipitated by calcium salts in a solution of low salt content.

Thrombosis (p. 114): Formation of a thrombus, a plug or clot, in a blood vessel or in one of the cavities of the heart, formed by the coagulation of the blood.

Thrombus (p. 132): See thrombosis.

Tissue Fibrinogen (pp. 53, 61, 63, 81): Wooldridge considered that the bulk of protoplasm consisted of tissue fibrinogens which interacted with fibrinogen-A and fibrinogen-B in blood to form fibrin. Tissue fibrinogen probably corresponds to thromboplastin. Wooldridge's theory was not susceptible to proof and was quickly abandoned.

Tissue Globulin (p. 53): Clot-accelerating substance isolated from tissues by Halliburton and Friend. Probably corresponds to thromboplastin.

Tissue Nucleoprotein (p. 53): Another synonym for a clot-promoting substance isolated from tissues. Probably thromboplastin.

Trypsin (p. 100): The main proteolytic enzyme of pancreatic secretion. It digests proteins to peptones and polypeptides through a series of reactions.

Tryptone (pp. 99, 110): Any peptone produced by the digestion of protein by trypsin. Also used in a more general sense to denote the products of pancreatic digestion.

Z

Zymoplastic Agent (pp. 30, 45, 50, 53, 60, 136): Term introduced by Alexander Schmidt. Refers to the clot-promoting substance(s) in tissues. Synonyms: thromboplastin, thrombokinase.

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*Note: In many instances the original bibliography of Morawitz was in*complete for modern library reference work or actually in error. For this reason the entire bibliography was checked and revised or corrected where necessary. Morawitz translated all non-German titles into German. We have given the titles in the original language. An asterisk (*) denotes the few instances in which it was impossible to verify a reference.

The translators are indebted to Mrs. Robert C. Hartmann, Mrs. Paul F. Guenther, and to Miss Eleanor Steinke and her staff at the Vanderbilt *Medical Library for undertaking the laborious task of revising the bib*liography. The staff of the National Library of Medicine kindly checked a number of references that were extremely difficult to verify.

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